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(54) Title: COMPOSITIONS AND METHODS FOR CONTROLLED DELIVERY OF VIRUS VECTORS

(57) Abstract

The invention relates to compositions and methods for delivering a virus vector to an animal. The compositions include compositions which comprise a matrix having a virus vector bound at the exterior surface thereof in a physiologically reversible manner. The invention also includes methods of making such compositions, including particles, devices, bulk materials, and other objects which comprise, consist of, or are coated with such compositions. Methods of delivering a virus vector to an animal tissue are also described.

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COMPOSITIONS AND METHODS FOR CONTROLLED DELIVERY OF VIRUS VECTORS

BACKGROUND OF THE INVENTION

Gene therapy is generally understood to refer to techniques designed to deliver nucleic acids, including antisense DNA and RNA, ribozymes, viral genome fragments and functionally active therapeutic genes into targeted cells (Culver, 1994, Gene Therapy: A Handbook for Physicians, Mary Ann Liebert, Inc., New York, NY). Such nucleic acids can themselves be therapeutic, as for example antisense DNAs that inhibit mRNA translation, or they can encode, for example, therapeutic proteins that promote, inhibit, augment, or replace cellular functions.

A serious shortcoming of current gene therapy strategies, including both *ex vivo* and *in vivo* gene therapy methods, is the inability of previously described vector and delivery system combinations to deliver nucleic acids efficiently into the interior of cells of a targeted population. In December, 1995, the U.S. National Institutes of Health issued a "Report and Recommendations of the Panel to Assess the NIH Investment in Research on Gene Therapy" (Orkin et al., 1995, National Institutes of Health, Bethesda, MD). In this Report, it was recognized that the development of gene therapy approaches to disease treatment was being inhibited, in part, by a dearth of effective gene transfer vectors. The Report recognized a need for further research applied to improving vectors for gene delivery.

Virus vectors are generally regarded as the most efficient nucleic acid delivery vectors. Recombinant replication-defective virus vectors have been used to transduce (i.e., infect or transfect) animal cells both *in vitro* and *in vivo*. Such vectors have included retrovirus, adenovirus, adeno-associated virus vectors, and herpes virus vectors. Although they are highly efficient for gene transfer, a major disadvantage associated with the use of virus vectors is the inability of many virus vectors to infect non-dividing cells. Another serious problem associated with the use of virus gene vectors is the potential for such vectors to induce an immune response in a patient to

whom they are administered. Such an immune response limits the effectiveness of the virus vector, since the patient's immune system rapidly clears the vector upon repeated or sustained administration of the vector. Furthermore, insertion of a gene into the genome of a cell by a virus vector can induce undesirable mutations in the cell. Other problems associated with virus gene vectors include inability to appropriately regulate gene expression over time in transfected cells, toxicity and other side effects caused by delivery of virus vectors to human tissues (e.g. liver damage and myocarditis), and potential production and transmission to other humans of harmful virus particles.

Furthermore, virus gene vectors, as used in prior art methods, have the drawback that they often cannot be delivered to a selected tissue in a specific, localized manner. Instead, many prior art methods of administering virus vectors result in vector being dispersed systemically or to tissues which adjoin, or are in fluid communication with, the desired target tissue. The inability of such methods to localize virus vector reduces the utility of the methods, because non-localized virus vector can transfect unintended tissues, elicit immune responses, be rapidly excreted from the body, or otherwise suffer diminished transfection ability. A significant need exists for methods of delivering virus vectors in a localized manner.

Virus vectors are able, to a limited degree, to deliver proteins and other therapeutic molecules to the cells which the virus vectors transfect. Such proteins and other therapeutic molecules can be incorporated passively and non-specifically into virus vector particles. Alternatively, as is known in the art, certain virus vectors specifically incorporate fusion proteins comprising a protein having a polypeptide viral packaging signal fused therewith.

Even though virus vectors have been widely used in experimental gene therapy protocols and human studies (Feldman et al., 1997, Cardiovasc. Res. 35:391-404; Roth et al., 1997, J. Natl. Cancer Inst. 89:21-39), none of these vectors has proven efficacious for virus vector-mediated gene therapy. It has been hypothesized that the shortcomings of adenovirus vectors has been due, at least in part, to limited transgene expression resulting from the immune response of the host individual and to cytotoxic effects which the vectors have exhibited toward organs of the host individual (Smith et al., 1996, Gene Ther. 3:190-200; Tripathy et al., 1996, Nat. Med. 2:545-549;

Nabel et al., 1995, Gene Ther. Cardiovasc. Dis. 91:541-548). Others working in the field have concentrated their efforts on mutating adenovirus vectors to render them relatively less immunogenic and toxic.

In addition to the low efficiency of virus vector uptake exhibited by most cell types and low levels of expression of the gene constructs delivered by virus vectors, many targeted cell populations are found in such low numbers in the body that the efficiency of transfection of these specific cell types is even further diminished. A critical need remains for gene therapy methods which can be used to efficiently deliver virus vectors to targeted cell populations. Others working in the field have concentrated on attempting to specifically target adenovirus vectors to a particular cell type, for example by attaching a specialized receptor ligand to the vectors (Tzimagiorgis et al., 1996, Nucl. Acids 24:3476-3477).

A virus vector useful for gene delivery must be delivered to its target cells in a form in which the biochemical components of the virus retain their function. That is, the virus vector must retain the capacity to bind to target cells, to transfer a nucleic acid carried by the vector into the interior of the cell, and, in some circumstances, to catalyze chemical reactions involving that nucleic acid within the cell (e.g. reverse transcription, integration into the host cell genome, or promoting transcription of gene elements on the nucleic acid). Thus, it is important that the delivery vehicle by means of which the virus vector is administered to a patient not subject the vector to chemically harsh or biochemically inactivating conditions. Thus, many matrices are not compatible for contacting with virus vectors. Ideally, a matrix in or on which a virus vector is disposed should be biodegradable, and in a form which is amenable to use in relevant surgical and therapeutic interventions. Further complicating matters, the following physiological phenomena are some of those which can inhibit administration of a virus vector to an animal tissue.

- Inability of the virus vector to interact specifically with cells of the desired tissue attributable to proteolytic degradation of one or more components of the virus vector by an enzyme in the animal.

- Complexing of animal proteins or other molecules with one or more components of the virus vector, with the result that the virus vector is unable to interact specifically with cells of the desired tissue.
- Sequestration of the virus vector in undesired tissues or organs of the animal (e.g. removal of virus vectors from the bloodstream by the liver).
- Complications (e.g. immune reactions, inappropriate transfection, rapid clearance of vector from the subject, etc.) arising from non-localized delivery of the virus vector.
- Inability of the virus vector to cross a physical barrier (e.g. the blood-brain barrier or peritoneal membranes) which separates the desired tissue from the site of administration of the virus vector.
- Induction of an immune response in the animal which results in production in the animal of cells and proteins (e.g. antibodies) which inactivate the virus vector.
- Relatively short duration of the period during which the virus vector contacts the desired tissue, either due to the immune response described above or due to rapid interaction of all available virus vector particles with the desired tissue.

A desirable virus vector will permit administration that is not significantly inhibited by these phenomena.

Others have demonstrated enhancement of transfection effected by combining adenovirus vectors with polylysine or cationic lipids to form soluble virus vector complexes (Fasbender et al., 1997, J. Biol. Chem. 272:6479-6489). However, the virus complexes still exhibit many of the disadvantages described herein which are characteristic of virus vectors, including a short duration of the period during which the virus vector is available to contact the desired tissue.

Others have attempted to deliver charged bioactive agents to biological systems by reversibly binding the charged bioactive agents to oppositely charged electrode surfaces, contacting the electrodes with the biological system, and thereafter relieving the charge on the electrode surface (e.g. U.S. Patents 4,585,652 and 5,208,154). Such compositions are severely limited by the necessity that electrical leads connect the electrodes to a power source and by the difficulty of effecting

sustained release of the bioactive agent from the electrode surface. For these reasons, such compositions are of severely limited usefulness for delivery of virus vectors to specific tissues.

A critical need remains for compositions which can be used to deliver virus vectors to desired tissues in a manner in which the period during which the vectors are administered is prolonged, immunogenicity associated with vector administration is minimized, and specificity of delivery of the vectors to desired tissues is maximized. At the same time, such compositions should preferably not adversely affect the biological activity of the virus vector (e.g. the efficiency with which the vector transfects cells) or of a nucleic acid contained within the vector. The compositions and methods described herein satisfy this need.

BRIEF SUMMARY OF THE INVENTION

The invention relates to a composition for delivery of a virus vector to an animal cell. The composition comprises the virus vector and a matrix having an exterior surface. The virus vector is bound at the exterior surface in a physiologically reversible manner. In one embodiment, the matrix is biodegradable and has an internal portion comprising the virus vector bound to the matrix in a physiologically reversible manner. In another embodiment, the virus vector is present substantially only at the exterior surface. In yet another embodiment, the matrix is not biodegradable and wherein the virus vector is bound to the matrix in a physiologically reversible manner.

Preferably, the composition described herein further comprises a virus-binding agent at the exterior surface, and the virus vector is bound to the virus-binding agent. In one embodiment, the matrix is biodegradable and has an internal portion comprising the virus vector bound to the virus-binding agent in a physiologically reversible manner. In an important embodiment, the virus vector has a charge, and wherein the virus-binding agent has a second charge opposite the charge of the virus vector.

The virus-binding agent of the composition described herein can, for example, be selected from the group consisting of a polycation, a polyanion, a cross-linking compound, a polypeptide which specifically binds with the virus vector, the

protein of a specifically-binding protein-ligand pair, and the ligand of a specifically-binding protein-ligand pair. The polycation can, for example, be selected from the group consisting of polylysine, polyarginine, polyornithine, polyhistidine, myelin basic protein, a low molecular weight glycopeptide, a cationic amphiphilic alpha-helical oligopeptide having a repeating sequence, a histone, a galactosylated histone, polybrene, spermine, spermidine, prolamine, polyethylenimine, putrescine, cadaverine, and hexamine. Preferably, polycation is poly-L-lysine. The polyanion can, for example, be a nucleic acid. The cross-linking compound can, for example, be selected from the group consisting of a di-sulfhydryl compound, SPDP, a di-aldehyde compound, and glutaraldehyde. The polypeptide which specifically binds with the virus vector can, for example, be selected from the group consisting of an antibody which specifically binds with the virus vector, a fragment of an antibody which specifically binds with the virus vector, and a virus receptor protein. The specifically-binding protein-ligand pair can, for example, be selected from the group consisting of biotin and an avidin, an antibody and an epitope to which the antibody specifically binds, and a viral coat protein and a cell-surface molecule with which the viral coat protein specifically binds.

In the composition described herein, the matrix can comprise a wide variety of materials, such as one or more selected from the group consisting of a charged biocompatible material, a biocompatible polymer, a biodegradable polymer, a biocompatible biodegradable polymer, polylactic acid, polyglycolic acid, polycaprolactone, a copolymer of polylactic acid and polyglycolic acid, a copolymer of polylactic acid and polycaprolactone, a copolymer of polyglycolic acid and polycaprolactone, a polygylcolide, a polyanhydride, a polyacrylate, a polyalkyl cyanoacrylate, n-butyl cyanoacrylate, isopropyl cyanoacrylate, a polyacrylamide, a polyorthoester, a polyphosphazene, a polypeptide, a polyurethane, a polystyrene, a polystyrene sulfonic acid, a polystyrene carboxylic acid, a polyalkylene oxide, a polyethylene, a polyvinyl chloride, a polyamide, a nylon, a polyester, a rayon, a polypropylene, a polyacrylonitrile, an acrylic, a polyisoprene, a polybutadiene, a polybutadiene-polyisoprene copolymer, a neoprene, a nitrile rubber, a polyisobutylene, an olefinic rubber, an ethylene-propylene rubber, an ethylene-propylene-diene

monomer rubber, a polyurethane elastomer, a silicone rubber, a fluoroelastomer, a fluorosilicone rubber, a vinyl acetate homopolymer, a vinyl acetate copolymer, an ethylene vinyl acetate copolymer, an acrylates homopolymer, an acrylates copolymer, polymethylmethacrylate, polyethylmethacrylate, polymethacrylate, ethylene glycol dimethacrylate, ethylene dimethacrylate, hydroxymethyl methacrylate, a polyvinylpyrrolidone, a polyacrylonitrile butadiene, a polycarbonate, a polyamide, a fluoropolymer, polytetrafluoroethylene, polyvinyl fluoride, a polystyrene, a styrene acrylonitrile homopolymers, a styrene acrylonitrile copolymer, a cellulose acetate, an acrylonitrile butadiene styrene homopolymer, a acrylonitrile butadiene styrene copolymer, a polymethylpentene, a polysulfone, a polyester, a polyimide, a polyisobutylene, a polymethylstyrene, an alginate, an agarose, a dextrin, a dextran, a multi-block polymer, a biocompatible metal alloy, titanium, platinum, stainless steel, hydroxyapatite, tricalcium phosphate, cocoa butter, a wax, and a ceramic material. Preferably the matrix comprises a biodegradable polymer such as a polylactate/polyglycolate copolymer.

In one embodiment of the composition described herein, the matrix is not electrically conductive.

The matrix of the composition described herein can have any of a variety of forms, such as one or more selected from the group consisting of a bulk material, a particle, a microsphere, a nanosphere, a device, a coating on a surface of a bulk material, a coating on a surface of a particle, a coating on a surface of a microsphere, a coating on a surface of a nanosphere, and a coating on a surface of a device.

In an important embodiment of the composition described herein, the virus vector comprises a transfection indicator. The transfection indicator can, for example, be selected from the group consisting of a nucleic acid, a nucleic acid analog, a transcription construct, an antisense oligonucleotide, a ribozyme, and an expression construct. For example, the transfection indicator can selected from the group consisting of an expression construct encoding a wound healing therapeutic protein, an expression construct encoding an anti-restenotic protein, an expression construct encoding an anti-oncogenic protein, an anti-restenotic antisense oligonucleotide, and an

anti-oncogenic antisense oligonucleotide. The wound healing therapeutic protein can, for example, be selected from the group consisting of TGF-β, FGF, PDGF, PDGF-β, IGF, M-CGF, BMP, GH, and PTH. The anti-restenotic protein can, for example, be selected from the group consisting of TPA, TGF-β, FGF, Rb, p21, and TK. The anti-oncogenic protein can, for example, be one encoded by a gene selected from the group consisting of *abl*, *akt2*, *apc*, *bcl2a*, *bcl2β*, *bcl3*, *bcr*, *brca1*, *brca2*, *cbl*, *ccnd1*, *cdk4*, *crk-II*, *csf1r/fms*, *dbl*, *dcc*, *dpc4/smad4*, *e-cad*, *e2f1/rbap*, *egfr/erbb-1*, *elk1*, *elk3*, *eph*, *erg*, *ets1*, *ets2*, *fer*, *fgr/src2*, *fli1/ergb2*, *fos*, *fps/fes*, *fra1*, *fra2*, *fyn*, *hck*, *hek*, *her2/erbb-2/neu*, *her3/erbb-3*, *her4/erbb-4*, *hras1*, *hst2*, *hstf1*, *ink4a*, *ink4b*, *int2/fgf3*, *jun*, *junb*, *jund*, *kip2*, *kit*, *kras2a*, *kras2b*, *lck*, *lyn*, *mas*, *max*, *mcc*, *met*, *mlh1*, *mos*, *msh2*, *msh3*, *msh6*, *myb*, *myba*, *mybb*, *myc*, *mycl1*, *mycn*, *nf1*, *nf2*, *nras*, *p53*, *pdgfb*, *pim1*, *pms1*, *pms2*, *ptc*, *pten*, *raf1*, *rb1*, *rel*, *ret*, *ros1*, *ski*, *src1*, *tal1*, *tgfbr2*, *thra1*, *thrb*, *tiam1*, *trk*, *vav*, *vhl*, *waf1*, *wnt1*, *wnt2*, *wt1*, and *yes1*. The anti-restenotic antisense oligonucleotide can, for example, be selected from the group consisting of a c-*myb* antisense oligonucleotide, a c-*myc* antisense oligonucleotide, and a PCNA antisense oligonucleotide. The anti-oncogenic antisense oligonucleotide can, for example, be selected from the group consisting of an *abl* antisense oligonucleotide, an *akt2* antisense oligonucleotide, an *apc* antisense oligonucleotide, a *bcl2a* antisense oligonucleotide, a *bcl2β* antisense oligonucleotide, a *bcl3* antisense oligonucleotide, a *bcr* antisense oligonucleotide, a *brca1* antisense oligonucleotide, a *brca2* antisense oligonucleotide, a *cbl* antisense oligonucleotide, a *ccnd1* antisense oligonucleotide, a *cdk4* antisense oligonucleotide, a *crk-II* antisense oligonucleotide, a *csf1r/fms* antisense oligonucleotide, a *dbl* antisense oligonucleotide, a *dcc* antisense oligonucleotide, a *dpc4/smad4* antisense oligonucleotide, an *e-cad* antisense oligonucleotide, an *e2f1/rbap* antisense oligonucleotide, an *egfr/erbb-1* antisense oligonucleotide, an *elk1* antisense oligonucleotide, an *elk3* antisense oligonucleotide, an *eph* antisense oligonucleotide, an *erg* antisense oligonucleotide, an *ets1* antisense oligonucleotide, an *ets2* antisense oligonucleotide, an *fer* antisense oligonucleotide, an *fgr/src2* antisense oligonucleotide, an *fli1/ergb2* antisense oligonucleotide, an *fos* antisense oligonucleotide, an *fps/fes* antisense oligonucleotide, an *fra1* antisense oligonucleotide, an *fra2* antisense oligonucleotide, an *fyn* antisense oligonucleotide, an *hck* antisense

oligonucleotide, an *hek* antisense oligonucleotide, an *her2/erbb-2/neu* antisense oligonucleotide, an *her3/erbb-3* antisense oligonucleotide, an *her4/erbb-4* antisense oligonucleotide, an *hras1* antisense oligonucleotide, an *hst2* antisense oligonucleotide, an *hstf1* antisense oligonucleotide, an *ink4a* antisense oligonucleotide, an *ink4b* antisense oligonucleotide, an *int2/fgf3* antisense oligonucleotide, a *jun* antisense oligonucleotide, a *junb* antisense oligonucleotide, a *jund* antisense oligonucleotide, a *kip2* antisense oligonucleotide, a *kit* antisense oligonucleotide, a *kras2a* antisense oligonucleotide, a *kras2b* antisense oligonucleotide, an *lck* antisense oligonucleotide, an *lyn* antisense oligonucleotide, an *mas* antisense oligonucleotide, an *max* antisense oligonucleotide, an *mcc* antisense oligonucleotide, an *met* antisense oligonucleotide, an *mlh1* antisense oligonucleotide, an *mos* antisense oligonucleotide, an *msh2* antisense oligonucleotide, an *msh3* antisense oligonucleotide, an *msh6* antisense oligonucleotide, an *myb* antisense oligonucleotide, an *myba* antisense oligonucleotide, an *mybb* antisense oligonucleotide, an *myc* antisense oligonucleotide, an *mycl1* antisense oligonucleotide, an *mycn* antisense oligonucleotide, an *nf1* antisense oligonucleotide, an *nf2* antisense oligonucleotide, an *nras* antisense oligonucleotide, a *p53* antisense oligonucleotide, a *pdgfb* antisense oligonucleotide, a *pim1* antisense oligonucleotide, a *pms1* antisense oligonucleotide, a *pms2* antisense oligonucleotide, a *ptc* antisense oligonucleotide, a *pten* antisense oligonucleotide, an *raf1* antisense oligonucleotide, a *rb1* antisense oligonucleotide, an *rel* antisense oligonucleotide, an *ret* antisense oligonucleotide, an *ros1* antisense oligonucleotide, an *ski* antisense oligonucleotide, an *src1* antisense oligonucleotide, a *tal1* antisense oligonucleotide, a *tgfbr2* antisense oligonucleotide, a *thra1* antisense oligonucleotide, a *thrb* antisense oligonucleotide, a *tiam1* antisense oligonucleotide, a *trk* antisense oligonucleotide, a *vav* antisense oligonucleotide, a *vhl* antisense oligonucleotide, a *waf1* antisense oligonucleotide, a *wnt1* antisense oligonucleotide, a *wnt2* antisense oligonucleotide, a *wt1* antisense oligonucleotide, and a *yes1* antisense oligonucleotide.

In another aspect of the composition described herein, the virus vector is selected from the group consisting of an adenovirus vector, a retrovirus vector, an adeno-associated virus vector, and a herpes virus vector. Preferably, the virus vector is an adenovirus vector.

The invention also relates to a surface coated with the composition described herein, and to an implantable device having a surface coated with a composition comprising a virus vector and a matrix having an exterior surface. The virus vector is bound at the exterior surface in a physiologically reversible manner. The device can, for example, be selected from the group consisting of a wound dressing, a suture, a particle, a vascular stent, and a bulk material.

When the device is a vascular stent, the matrix is preferably a polylactate/polyglycolate copolymer comprising polylysine at the exterior surface, wherein the virus vector is bound with the polylysine, and the virus vector preferably comprises a transfection indicator selected from the group consisting of an expression construct encoding an anti-restenotic protein and an anti-restenotic antisense oligonucleotide.

When the device is a suture, the matrix is preferably a polylactate/ polyglycolate copolymer comprising polylysine at the exterior surface, wherein the virus vector is bound with the polylysine, and the virus vector preferably comprises an expression construct encoding a wound healing protein.

When the device is a particle, the matrix is preferably a polylactate/ polyglycolate copolymer comprising polylysine at the exterior surface, wherein the virus vector is bound with the polylysine, and the virus vector preferably comprises a transfection indicator selected from the group consisting of an expression construct encoding a wound healing therapeutic protein, an expression construct encoding an anti-restenotic protein, an expression construct encoding an anti-oncogenic protein, an anti-restenotic antisense oligonucleotide, and an anti-oncogenic antisense oligonucleotide. Particles preferably have a diameter no greater than about 900 micrometers, and more preferably no greater than about 1 micrometer.

When the device is a bulk material, the matrix is preferably a polylactate/polyglycolate copolymer comprising polylysine at the exterior surface, wherein the virus vector is bound with the polylysine, and the virus vector preferably comprises a transfection indicator selected from the group consisting of an expression construct encoding a wound healing therapeutic protein, an expression construct encoding an anti-restenotic protein, an expression construct encoding an anti-oncogenic

protein, an anti-restenotic antisense oligonucleotide, and an anti-oncogenic antisense oligonucleotide.

The invention also relates to a method of making a composition for delivery of a virus vector to an animal. This method comprises contacting the virus vector with a matrix which has an exterior surface. The virus vector binds at the exterior surface in a physiologically reversible manner. The matrix preferably comprises a virus-binding agent at the exterior surface, and is preferably biodegradable. The matrix can, for example, be on a surface of an implantable device.

When the matrix is on a surface of an implantable device, it can be put there by applying a suspension comprising the matrix and a solvent to the surface of the implantable device and at least substantially removing the solvent from the surface prior to contacting the virus vector with the matrix. Preferably, the suspension further comprises the virus-binding agent. In another embodiment, a precursor composition comprising a plurality of monomers of the polymer is applied to the surface of the implantable device and the monomers are polymerized prior to contacting the virus vector with the matrix. The precursor composition can, optionally, further comprises the virus-binding agent.

The invention further relates to a method of delivering a virus vector to an animal tissue. This method comprises placing in fluid communication with the animal tissue a composition comprising a matrix having an exterior surface. The virus vector is bound at the exterior surface in a physiologically reversible manner. In one embodiment of this method, the composition further comprises a virus-binding agent at the exterior surface, and the virus vector is bound to the virus-binding agent. Preferably, the composition is placed in contact with the animal tissue. The animal tissue can be outside of the body of the animal from which it was obtained, or alternatively, it can be in the same or a different animal.

The composition can be placed in fluid communication with the tissue by placing in fluid communication with the tissue an implantable device having a surface coated with the composition. For example, the device can be selected from the group consisting of a wound dressing, a suture, a particle, a vascular stent, and a bulk material.

The tissue which is contacted with the composition can, for example, be selected from the group consisting of a wounded tissue, an ischemic tissue, a gastrointestinal tissue, an embryonic tissue, and a fetal tissue. Preferably, the animal is a human.

The invention still further relates to a kit comprising a matrix having an exterior surface, a virus-binding agent, and an instructional material which describes binding a virus vector at the exterior surface in a physiologically reversible manner. Optionally, the kit can further comprise a virus-binding agent. The virus-binding agent is preferably present at the exterior surface. Alternatively, the kit can further comprise a virus vector precursor, such as an adenovirus vector comprising a nucleic acid having a multiple restriction site.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1, comprising Figures 1A, 1B, 1C, 1D, 1E, and 1F is a series of images. Figures 1A and 1B are a pair of images which depict the immunofluorescent microscopic appearance of the cationic polymer substrate described herein which was contacted with a buffer in which fluorescently labeled, negatively charged polystyrene nanoparticles were suspended (Figure 1A) or with the same buffer which did not comprise the nanoparticles (Figure 1B). The objective magnification in each of these two images was 20×. Figures 1C through 1F are a quartet of images which depict the phase contrast microscopic appearance of X-gal A10 vascular smooth muscle cells which were cultured in the presence of a composition comprising a matrix having virus vectors which comprised (Figures 1C and 1D) or did not comprise (Figures 1E and 1F) an expression construct encoding β-galactosidase. The cells were incubated for one day (Figures 1C and 1E) or for four days (Figures 1D and 1F) in the presence of the composition prior to X-gal staining and imaging. The objective magnification in each of these four images was 10×.

DETAILED DESCRIPTION

The invention is based on the discovery that sustained-release delivery of a virus vector can be improved by providing a composition comprising a matrix

having a virus-binding exterior surface having the virus vector bound thereto in a physiologically reversible manner. The compositions and methods described herein are particularly useful for delivery of a nucleic acid to a cell or tissue of an animal.

Compared to prior art virus vectors, the compositions described herein, which comprise a virus vector, exhibit reduced immunogenicity and enhanced delivery of the virus vector to desired cells. Without wishing to be bound by any particular theory of operation, it is believed that the compositions described herein minimize immunogenicity elicited by the virus vector by immobilizing the vector at the surface of the composition, thereby minimizing contact between the vector and components of the host immune system. Further without wishing to be bound by any particular theory of operation, it is believed that the composition described herein enhances gene delivery to a target tissue by providing sustained release of the virus vector geometrically close to the target tissue.

The sustained virus vector release properties of the composition described herein permit long-term fetal exposure to the virus vector of the composition during the period during which fetal immune competence is developed. Thus, the composition described herein can be used to effect *in utero* immune tolerization of an animal such as a human to a virus vector such as an adenovirus vector. By making an animal having an immune system which tolerates the virus vector of the composition, one is enabled to perform gene therapy upon the animal using the virus vector, *in utero*, in a neonatal animal, in a juvenile animal, or in an adult animal.

The compositions described herein comprise a virus vector and a matrix. The matrix has an exterior surface, and the virus vector is bound at the exterior surface of the matrix in a physiologically reversible manner. In one embodiment, a virus-binding agent having a charge opposite that of the virus vector is present at the exterior surface of the matrix, and at least most of the virus vector is bound to the virus-binding agent. The virus vector can be bound to the virus-binding agent in either a physiologically reversible manner or in a non-physiologically reversible manner. When the virus vector is bound to the virus-binding agent in a non-physiologically manner, it is necessary either that the matrix be biodegradable or that the virus-binding agent be bound to the exterior surface of the matrix in a physiologically-reversible

manner, in order to permit dissociation of the virus vector from the matrix. If the virus vector is bound to the virus-binding agent in a physiologically reversible manner, then it does not matter whether the matrix is biodegradable or whether the virus-binding agent is bound to the matrix in a physiologically reversible manner. In an important embodiment of the composition described herein, the matrix is itself a virus-binding agent, in which case it is not necessary to use a virus-binding agent other than the matrix. In another important embodiment, the virus-binding agent is an antibody linked with the matrix, wherein the antibody specifically binds with an epitope on the surface of the virus vector. In the presence of human physiological ion concentrations, virus vector specifically bound by the antibody dissociates from the antibody, and thus from the matrix.

In other important embodiments, the composition described herein coats a surface, such as a surface of an implantable device. Such surfaces and devices comprise the matrix at a surface, and have the virus vector bound to the exterior surface of the matrix in a physiologically reversible manner. Upon implantation of the device, the virus vector is released in the vicinity of the device. The device can be one made solely for the purpose of delivering the virus vector (e.g. particles or bulk materials comprising the composition), or it can be a device having a separate physiological function (e.g. wound dressings or vascular stents comprising the composition).

In another important embodiment of the composition described herein, the matrix comprises a biodegradable polylactic-polyglycolic acid (PLGA) copolymer which has polylysine either suspended therein or bound to the exterior surface thereof. A negatively charged virus vector such as an adenovirus vector is bound to the polylysine. Upon biodegradation of the PLGA copolymer, virus vectors, virus vector/polylysine complexes, or both, are released from the matrix. Delivery of the virus vector to a tissue in fluid communication with the composition is thereby effected. Preferably, the composition is placed in contact with the tissue to which it is desired to deliver the virus vector.

The compositions described herein are particularly amenable to use on implantable devices. The devices can be fashioned from or coated with a composition described herein. In various embodiments:

- the matrix is used to form or to coat a wound dressing (e.g. a bandage or a hernia repair mesh), and the virus vector comprises an expression construct encoding a wound healing therapeutic protein;
- the matrix is used to form or to coat a vascular stent, and the virus vector comprises either an expression construct encoding an anti-restenotic protein or an anti-restenotic oligonucleotide;
- the matrix is used to form or to coat a suture, and the virus vector comprises an expression construct encoding a wound healing therapeutic protein; or
- the matrix is used to form or to coat a particle or a bulk material, and the virus vector comprises either an expression construct encoding an anti-oncogenic protein or an anti-oncogenic antisense oligonucleotide.

These embodiments are, of course, merely non-limiting examples of how the compositions described herein can be used to deliver a virus vector to cells or tissues in an animal.

When the virus vector comprises an expression construct, it is preferred that the expression construct be targeted to the nucleus. Nuclear targeting can, for example, be achieved by attaching a protein to the surface of a virus using a conjugated virus-specific antibody. This method has been demonstrated using an antibody linked to basic fibroblast growth factor (bFGF), wherein the antibody binds specifically with an adenovirus. Nuclear targeting of bFGF in this manner has been demonstrated by others to enhance expression through both an FGF receptor entry mechanism and enhanced nuclear entry. Nuclear entry of plasmid DNA and viruses can also be enhanced using polymers or specific protein and peptide sequences, as described in the art (Pollard et al., 1998, J. Biol. Chem. 273:7507-7511; Sebestyen et al., 1998, Nature Biotechnol. 16:80-85; Neill et al., 1995, Biochem. Soc. Trans. 23:346S; Peeples et al., 1992, J. Virol. 66:3263-3269).

As described herein, the components of the compositions of the invention, including the matrix, the virus vector, and the virus-binding agent (if different from the matrix) can be varied quite liberally. It is preferred to optimize the composition prior to its use *in vivo* or *ex vivo* to effect delivery of the virus vector to one or more cells or tissues of an animal. The process of optimizing the compositions

can be performed using an *in vitro* model of the cells to which the virus vector is to be delivered or an *in vivo* model of the cells in an animal of a species different than the species to which the virus vector is ultimately to be delivered. It is important that the virus vector be capable of transfecting both cells in the model system and the cells in the animal to which the virus vector is ultimately to be delivered. Methods of selecting cells which are susceptible to transfection with a virus vector, as well as methods of testing whether cells are susceptible to transfection with a virus vector are known in the art, and can be used to select appropriate model cells. Preferably, the model cells are of the same type and the same species as the cells to which the virus vector is ultimately to be delivered. *In vitro* or *in vivo* experiments to test various components of the composition described herein, methods of preparing such compositions, and the like, can be performed using the appropriately selected model cells in order to identify an optimal composition prior to administration of that composition to the desired animal. By way of example, model cells which are useful for optimizing compositions for delivery of a virus vector to vascular tissues include primary human fibroblasts in *in vitro* culture, 293 kidney epithelial cells in *in vitro* culture, and A10 vascular smooth muscle cells in *in vitro* culture.

The means by which physiologically reversible binding of the virus vector to the exterior surface of the matrix is effected is not critical. For example, the virus vector can be directly bound to the matrix, or it can be bound to a virus-binding agent which is a component of the matrix, coats the matrix, or is bound to at least the exterior surface of the matrix. After binding the virus vector to the exterior surface of the matrix, the composition can optionally be coated with or embedded in a biodegradable material, although it preferably is not.

Physiological reversibility of virus vector binding to the matrix can be effected in several ways. If the virus vector is charged, as are most virus vectors, the vector can be non-covalently bound to a matrix having the opposite charge or to a virus-binding agent having the opposite charge which is present at the exterior surface of the matrix. When placed into a physiological system, exchange of ions for the virus vector releases the virus vector from the surface. Similarly, if the virus vector comprises a protein or a ligand of a protein-ligand pair (e.g. an antibody-antigen pair),

the virus vector can be bound to the exterior of the matrix if the other member of the protein-ligand pair is present at the exterior surface of the matrix. When placed into a physiological system, the ion content of the physiological system, the presence in the system of a molecule which inhibits interaction of the protein and its ligand, or cleavage of a bond which links the protein or the ligand with either the matrix or the virus vector, effects releases the virus vector from the surface. By way of example, if an antibody is covalently linked to the matrix and a ligand comprising an epitope to which the antibody specifically binds is present on the surface of the virus vector, the virus vector can be bound to the matrix. If a molecule comprising the epitope is present in the physiological system to which the composition is to be delivered, then the molecule can interfere with binding of the antibody and its ligand, thereby effecting release of the vector from the matrix. If the matrix is biodegradable in the physiological system to which the composition is to be delivered, then the virus vector can be covalently or non-covalently bound to either the matrix or a virus-binding agent which can be covalently or non-covalently bound to the matrix. Biodegradation of the matrix releases the virus vector, in the form of the virus vector alone, the virus vector linked with at least a portion of the matrix, or the virus vector linked with at least a portion of the virus-binding agent. Thus released from the matrix, the virus vector can transfect a cell in fluid communication with the matrix.

The virus vector of the compositions described herein is preferably present only at the exterior surface of the matrix. In certain embodiments, this exterior surface is ridged, porous, 'fuzzy' (i.e. having a multiplicity of thin projections), or otherwise irregular, although, of course, the exterior surface can also be smooth.

The virus vector can optionally be present within the matrix as well as at the exterior surface thereof. In one embodiment, a biodegradable matrix is laid down in discrete layers, the virus vector being bound at the exterior surface of the matrix at each layer prior to deposition of the next layer atop the exterior surface. In another embodiment, the virus vector is suspended throughout the matrix, and the virus vector present at the exterior surface of the matrix is bound in a physiologically reversible manner. According to this embodiment, the matrix can be biodegradable or not. If the matrix is not biodegradable, it does not matter whether virus vector not present at the

exterior surface of the matrix is bound in a physiologically reversible manner. However, if the matrix is biodegradable, then the virus vector not present at the exterior surface of the matrix is preferably bound to the matrix, or to a virus-binding agent in the matrix, in a physiologically reversible manner.

Exemplary materials which can be used in the compositions of the invention are now described. It is understood, however, that the materials described herein represent only non-limiting examples of materials which can be used. In light of the present disclosure, it will be evident to the skilled artisan how materials which are not specifically listed here, or which are hereafter developed or discovered, can be used to generate the composition described herein.

The Virus Vector of the Composition

The virus vector can be substantially any virus vector which can be bound at a surface in a physiologically reversible manner. By way of example, the virus vector can be an adenovirus vector, a retrovirus vector, an adeno-associated virus vector, or a herpes virus vector. In a preferred embodiment, the virus vector which is used in the compositions and methods described herein is an adenovirus vector. Adenovirus vectors are the most potent agents investigated thus far for gene therapy. However, their clinical use has been limited because of immunogenicity and toxicity concerns. In particular, a type 5 replication defective adenovirus vector comprising an expression construct which comprises a cytomegalovirus (CMV) promoter is preferred. Such vectors have been described by others (e.g. Adam et al., 1995, J. Gen. Virol. 76:3153-3157; Gonin et al., 1995, Vet. Microbiol. 45:393-401; Acsadi et al., 1994, Gene Ther. 1:338-340). The expression construct preferably encodes a therapeutic protein, such as human platelet derived growth factor β (PDGF-β) or a reporter protein, the presence of which can be conveniently assayed, such as *Escherichia coli* beta-galactosidase protein.

The nucleic acid carried by the virus vector in the present invention can be, by way of example and not limitation, an oligonucleotide or polynucleotide such as an antisense DNA molecule, an antisense RNA molecule, a catalytic RNA molecule or a catalytic RNA/protein complex (i.e. a "ribozyme"), an expression construct comprising a DNA molecule encoding a protein such as a therapeutic protein, a

transcribable construct comprising a DNA molecule encoding a ribozyme, a viral genome fragment such as a viral DNA or RNA molecule, an RNA molecule encoding a protein such as a therapeutic protein, a plasmid, a cosmid, a DNA molecule encoding a portion of the genome of an organism, a cDNA molecule, a gene fragment; or a DNA molecule in any of its superstructural forms, including single-stranded DNA, double stranded DNA, supercoiled DNA, triple-helical DNA, Z-DNA, and the like.

The nucleic acid of the virus vector can be prepared or isolated by any conventional means typically used to prepare or isolate nucleic acids. For example, DNA and RNA molecules can be chemically synthesized using commercially available reagents and synthesizers by methods that are known in the art (e.g., Gait, 1985, In: Oligonucleotide Synthesis: A Practical Approach, IRL Press, Oxford, England). RNA molecules can also be produced in high yield via *in vitro* transcription methods using plasmids such as SP65 (available from Promega Corporation, Madison, WI). The nucleic acids can be purified by any suitable means, as many such means are known in the art. For example, the nucleic acids can be purified by reverse-phase or ion exchange HPLC, size exclusion chromatography, or gel electrophoresis. Of course, the skilled artisan will recognize that the method of purification will depend in part on the size of the DNA to be purified. Nucleic acids suitable for delivery using a virus vector can also be prepared using any of the innumerable recombinant methods which are known or are hereafter developed.

Nucleic acids having modified internucleoside linkages can also be used in the virus vector described herein. Nucleic acids containing modified internucleoside linkages can be synthesized using reagents and methods that are known in the art. For example, methods for synthesizing nucleic acids containing phosphonate, phosphorothioate, phosphorodithioate, phosphoramidate methoxyethyl phosphoramidate, formacetal, thioformacetal, diisopropylsilyl, acetamidate, carbamate, dimethylene-sulfide ($-CH_2-S-CH_2-$), dimethylene-sulfoxide ($-CH_2-SO-CH_2-$), dimethylene-sulfone ($-CH_2-SO_2-CH_2-$), 2'-O-alkyl, and 2'-deoxy-2'-fluoro-phosphorothioate internucleoside linkages are known in the art (e.g. Uhlmann et al., 1990, Chem. Rev. 90:543-584; Schneider et al., 1990, Tetrahedron Lett. 31:335).

The nucleic acid can, for example, be a therapeutic agent, such as an antisense DNA molecule that inhibits mRNA translation. Alternatively, by way of example, the nucleic acid can encode a therapeutic agent, such as a transcription or translation product which, when expressed by a cell to which the nucleic acid-containing composition is delivered, has a favorable therapeutic effect upon the cell. Exemplary therapeutic transcription products include antisense RNA molecules, ribozymes, viral genome fragments, and the like. Exemplary therapeutic translation products include therapeutic proteins, such as membrane proteins, transcription factors, intracellular proteins, cytokine binding proteins, wound healing proteins, anti-restenotic proteins, anti-oncogenic proteins, and the like.

In a preferred embodiment of the invention, the nucleic acid of the virus vector is a DNA molecule that encodes gene products that stimulate or promote healing of wounded or damaged tissues in vivo or alleviate the symptoms of disease. Particularly preferred are therapeutic proteins, such as growth factors and hormones. Particularly preferred growth factors are transforming growth factor-beta (TGF-β; Cox, D.A., 1995, Cell Biology International 19: 357 -371), acidic fibroblast growth factor (FGF; Slavin, 1995, Cell Biol. Intl. 19:431-444), platelet derived growth factor (PDGF), insulin like growth factor (IGF), macrophage-colony stimulating factor (M-CSF), and bone morphogenic protein (BMP); particularly preferred hormones are human growth hormone (GH) and human parathyroid hormone (PTH).

In another preferred embodiment, the nucleic acid of the virus vector is selected from an expression construct encoding an anti-restenotic protein and an anti-restenotic antisense oligonucleotide. Exemplary anti-restenotic proteins include tissue plasminogen activator (TPA), TGF-β, FGF, retinoblastoma protein (Rb), p21, and thymidine kinase (TK). Exemplary anti-restenotic antisense oligonucleotides include a c-*myb* antisense oligonucleotide, a c-*myc* antisense oligonucleotide, and a PCNA antisense oligonucleotide.

In another preferred embodiment of the invention, the nucleic acid of the virus vector is selected from an expression construct encoding an anti-oncogenic protein and an anti-oncogenic antisense oligonucleotide. Exemplary anti-oncogenic proteins include those encoded by the following genes: *abl*, *akt2*, *apc*, *bcl2α*, *bcl2β*,

bcl3, *bcr*, *brca1*, *brca2*, *cbl*, *ccnd1*, *cdk4*, *crk-II*, *csf1r/fms*, *dbl*, *dcc*, *dpc4/smad4*, *e-cad*, *e2f1/rbap*, *egfr/erbb-1*, *elk1*, *elk3*, *eph*, *erg*, *ets1*, *ets2*, *fer*, *fgr/src2*, *fli1/ergb2*, *fos*, *fps/fes*, *fra1*, *fra2*, *fyn*, *hck*, *hek*, *her2/erbb-2/neu*, *her3/erbb-3*, *her4/erbb-4*, *hras1*, *hst2*, *hstf1*, *ink4a*, *ink4b*, *int2/fgf3*, *jun*, *junb*, *jund*, *kip2*, *kit*, *kras2a*, *kras2b*, *lck*, *lyn*, *mas*, *max*, *mcc*, *met*, *mlh1*, *mos*, *msh2*, *msh3*, *msh6*, *myb*, *myba*, *mybb*, *myc*, *mycl1*, *mycn*, *nf1*, *nf2*, *nras*, *p53*, *pdgfb*, *pim1*, *pms1*, *pms2*, *ptc*, *pten*, *raf1*, *rb1*, *rel*, *ret*, *ros1*, *ski*, *src1*, *tal1*, *tgfbr2*, *thra1*, *thrb*, *tiam1*, *trk*, *vav*, *vhl*, *waf1*, *wnt1*, *wnt2*, *wt1*, and *yes1*.

Modified gene sequences, i.e. genes having sequences that differ from the gene sequences encoding the native proteins, are also encompassed by the invention, so long as the modified gene still encodes a protein that exhibits the biological activity of the native protein, at a greater or lesser level of activity. These modified gene sequences include modifications caused by point mutations, modifications due to the degeneracy of the genetic code or naturally occurring allelic variants, and further modifications that are introduced by genetic engineering, i.e., by the hand of man, to produce recombinant nucleic acid molecules.

The nucleic acid of the virus vector described herein can be recombinantly engineered into a variety of known host vector systems that provide for replication of the nucleic acid on a large scale for the preparation of composition described herein. These vectors can be designed, using known methods, to contain the elements necessary for directing transcription, translation, or both, of the nucleic acid. Methods which are known to the skilled artisan can be used to construct expression constructs having the protein coding sequence operably linked with appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques and synthetic techniques (see, e.g. Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York; Ausubel et al., 1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York).

The nucleic acid encoding the protein(s) of interest can be operatively associated with a variety of different promoter/regulator sequences. The promoter/regulator sequences can be selected to optimize expression of therapeutic amounts of protein. In some instances, the promoter/regulator sequences can be

constitutive or inducible promoters and can be used under the appropriate conditions to direct high level or regulated expression of the gene of interest. Preferably, the nucleic acid encoding the protein(s) of interest is a CMV promoter.

It is also within the scope of the invention that the nucleic acid of the virus vector described herein contains a plurality of protein-coding regions, combined on a single genetic construct under control of one or more promoters, or prepared as separate constructs of the same or different types. Alternatively, the two or more protein-coding regions can be under the transcriptional control of a single promoter, and the transcript of the nucleic acid can comprise one or more internal ribosome entry sites interposed between the protein-coding regions. Thus, an almost endless combination of different genes and genetic constructs can be employed. Any and all such combinations are within the scope of the present invention.

The virus vector of the composition described herein can be used to deliver molecules other than nucleic acids to targeted cells. Examples of such other molecules include proteins, polyamines, and known pharmaceutical compositions. These molecules can be incorporated into the virus vector by generating the virus vectors in cells which comprise such molecules.

The Matrix of the Composition

The matrix of the composition described herein can be substantially any material onto at least the exterior surface of which the virus vector described herein can be bound in a physiologically reversible manner, either directly or by way of a virus-binding agent suspended in, incorporated within, linked to, or coating the matrix. Preferably, the matrix is biocompatible. Also preferably, the matrix comprises a polymeric material. Where the virus vector is bonded in a non-physiologically reversible manner to either the matrix or to a virus-binding agent suspended in, incorporated within, linked to, or coating the matrix, the matrix must be biodegradable in order to permit the virus vector to be released from the exterior surface thereof in a physiologically reversible manner following delivery of the composition.

A great many polymer materials are suitable for making the composition described herein. Depending on the manner in which delivery of the virus vector is contemplated, the polymeric matrix can be non-biodegradable, or, preferably,

biodegradable. The polymers can be a naturally-occurring polymer or a synthetic polymer. Discussion and a non-limiting list of suitable polymers can be found in Mathiowitz et al., P.C.T. publication number WO95/24929 (particularly at pages 6-9), and Goldstein et al., P.C.T. publication number WO97/47254 (particularly pages 22-35). Particularly preferred polymers for use in the compositions of the present invention include biocompatible biodegradable polymers such as polylactic acid, polyglycolic acid, polycaprolactone, and copolymers thereof. In preferred embodiments, the biocompatible biodegradable polymer is a copolymer of polylactic acid and polyglycolic acid (PLGA copolymer). Also preferably, the proportion of lactate monomers to glycolate monomers in the PLGA copolymer ranges from near infinity (i.e. the polymer comprises essentially only lactate monomers) to a lactate:glycolate ratio of about 25:75. More preferably, this ratio is about 50:50. It is understood that the higher the proportion of lactate monomers to glycolate monomers is, the less rapidly the copolymer will be biodegraded. Similarly, a copolymer having a relatively low lactate:glycolate monomer ratio (i.e. 50:50 or 25:75) will release the virus vector at a rate greater than the rate of a copolymer having a relatively high lactate:glycolate monomer ratio (i.e. 75:25 or 100:1). Other suitable biocompatible biodegradable polymers include polyesters such as polyglycolides, polyanhydrides, polyacrylates, polyalkyl cyanoacrylates such as n-butyl cyanoacrylate and isopropyl cyanoacrylate, polyacrylamides, polyorthoesters, polyphosphazenes, polypeptides, polyurethanes, polystyrenes, polystyrene sulfonic acid, polystyrene carboxylic acid, polyalkylene oxides, alginates, agaroses, dextrins, dextrans, and mixtures of such polymers.

The polymer used to form the matrix of the composition described herein can also be non-biodegradable, so long as the polymer is biocompatible. Examples of biocompatible non-biodegradable polymers which are useful in the compositions include polyethylenes, polyvinyl chlorides, polyamides such as nylons, polyesters, rayons, polypropylenes, polyacrylonitriles, acrylics, polyisoprenes, polybutadienes and polybutadiene-polyisoprene copolymers, neoprenes and nitrile rubbers, polyisobutylenes, olefinic rubbers such as ethylene-propylene rubbers, ethylene-propylene-diene monomer rubbers, and polyurethane elastomers, silicone

rubbers, fluoroelastomers and fluorosilicone rubbers, homopolymers and copolymers of vinyl acetates such as ethylene vinyl acetate copolymer, homopolymers and copolymers of acrylates such as polymethylmethacrylate, polyethylmethacrylate, polymethacrylate, ethylene glycol dimethacrylate, ethylene dimethacrylate and hydroxymethyl methacrylate, polyvinylpyrrolidones, polyacrylonitrile butadienes, polycarbonates, polyamides, fluoropolymers such as polytetrafluoroethylene and polyvinyl fluoride, polystyrenes, homopolymers and copolymers of styrene acrylonitrile, cellulose acetates, homopolymers and copolymers of acrylonitrile butadiene styrene, polymethylpentenes, polysulfones, polyesters, polyimides, polyisobutylenes, polymethylstyrenes, and other similar compounds known to those skilled in the art.

The use of multi-block polymers in the matrix of the composition described herein is contemplated. Such multi-block polymers can be biodegradable or not, and can be particularly useful to produce microspherical matrices, as described herein. Multi-block polymers comprise monomers of a plurality of types, wherein numerous monomers of a first type are polymerized and linked to a region comprising numerous polymerized monomers of a second type. Multi-block polymers can be used to facilitate production of microspheres, or to affect various properties of the microspheres. For example, subunits comprising an emulsifying agent can be incorporated into the multi-block polymer, obviating the need to add an emulsifying agent during production of microspheres using the polymer. Alternatively, hydrophobic or hydrophilic subunit blocks can be used. Suitable multi-block copolymers, as well as their uses, are described in Levy et al., P.C.T. publication number WO96/20698, and Goldstein et al., P.C.T. publication number WO97/47254.

In a particularly preferred embodiment of the composition described herein, the matrix of the composition is present in the form of microspheres or nanospheres. Preferably, the microspheres have a generally spherical shape and have a maximum physical dimension (analogous to the diameter of a sphere) of from about 1 micrometer to 900 micrometers, and more preferably from about 1 micrometer to 10 micrometers. Preferably, the nanospheres have a generally spherical shape and have a maximum physical dimension less than about 1 micrometer, more preferably less than

about 300 nanometers, and even more preferably from about 70 nanometers to about 160 nanometers. Coatings for implantable or injectable particles or devices which comprise the composition comprising the matrix in a microspherical or nanospherical form are contemplated. Such particles and devices have the advantage of releasing microspheres or nanospheres over time, wherein the microspheres or nanospheres release the virus vector on a sustained basis.

Microspherical and nanospherical matrices are preferred because these forms are known to be amenable to penetration into mammalian tissues and uptake into mammalian cells. Furthermore, the total surface area of a composition comprising a matrix in the form of microspheres or nanospheres is greater than the total surface area of a bulk form of the same volume of matrix, meaning that biodegradation and/or release of the virus vector from the surface of the matrix can be increased if microspheres or nanospheres are used, rather than a bulk form of the matrix. Although the use of microspheres and nanospheres for delivery of pharmaceutical agents has been described by others, the use of such delivery vehicles having a virus vector bound at the surface thereof in a physiologically reversible manner in order to provide the vector to a mammalian tissue or cells has not been described by others.

The polymers used in producing the microspheres can optionally have other molecules bound to them. These modifications can, for example, impart the microspheres with the ability to target and bind specific tissues or cells, allow them be retained at the administration site, exhibit anti-thrombogenic effects, prevent aggregation, and/or alter the release properties of the microspheres. Production of such modified polymers and surface-modified microspheres made from them are discussed in Levy et al., P.C.T. publication number WO96/20698, the disclosure of which is hereby incorporated by reference. The targeting agent can alternatively be bound to the virus-binding agent described herein. As a specific example, it can be desirable to incorporate receptor-specific molecules, such as antibodies, into or onto the microspheres to mediate receptor-specific particle uptake.

Non-polymeric materials which can be used as the matrix in the compositions described herein include any material onto the exterior surface of which the virus vector described herein can be bound in a physiologically reversible manner,

either directly or by way of a virus-binding agent suspended in, incorporated within, linked to, or coating the matrix. Such non-polymeric materials can be biodegradable or non-biodegradable, but must be biocompatible. By way of example, useful non-polymeric materials include titanium, platinum, stainless steel, other biocompatible metal alloys, hydroxyapatite, tricalcium phosphate, cocoa butter, waxes, and ceramic materials.

Other preferred materials useful as the matrix in the compositions described herein include a collagen, more preferably a type I collagen, such as human or bovine type I collagen, or another self-assembling biopolymer, such as a laminin, an elastin, a pro-elastin peptide, and the like.

The Virus-Binding Agent of the Composition

The chemical identity of the virus-binding agent of the composition described herein is not critical. Instead, it is important that the virus-binding agent bind the virus vector to the exterior surface of the matrix in a physiologically reversible manner. In one embodiment, the virus-binding agent and the matrix are the same material, the virus vector being bound directly to the exterior surface of the matrix in a physiologically reversible manner. In another embodiment, the virus-binding agent is a compound which has a charge that is opposite the charge of the virus vector of the composition and which is suspended in, incorporated within, linked to, or coats the matrix. Thus, when an anionic virus vector is used in the compositions and methods described herein, a cationic, and preferably a polycationic, virus-binding agent should be used. Similarly, when a cationic virus vector is used in the compositions and methods described herein, an anionic, and preferably a polyanionic, virus-binding agent should be used. When a virus vector having both positive and negative charges is used, the virus-binding agent should have a charge that is the opposite of the net charge of the vector. When a virus vector having a plurality of charged groups but having a net charge near neutrality is used, either an anionic or a cationic virus-binding agent can be used.

The suitability of a virus-binding agent for use in combination with a given virus vector can be determined by combining the virus-binding agent and the virus vector and assessing whether the virus vector binds with the virus-binding agent.

Such binding can be assessed using any known method including chromatographic, microscopic, and spectroscopic methods. For example, in one such method the virus-binding agent is linked with a surface, the surface is contacted with a suspension of the virus, rinsed with a solution which does not contain the virus, and contacted with a suspension of a detectably labeled antibody which binds specifically with the virus, and then the presence or absence of the detectable label at the surface is assessed. The presence of the detectable label at the surface is an indication that the virus-binding agent is useful for binding the virus. For example, when the virus vector is a recombinant type 5 adenovirus, the detection method used can be that described by Yee et al. (1985, Virology 147:142-153).

One preferred family of virus-binding agents is the polylysines. Polylysines consist of polypeptides of varying lengths, comprising (e.g. primarily or exclusively) lysine residues, which are positively charged at human physiological blood pH. The lysine residues can be D-lysine residues, L-lysine residues, or a mixture of the two enantiomers; poly-L-lysine is preferred. The polylysines which are useful as virus-binding agents in the compositions and methods described herein include all variants of polylysine, regardless of length, linear, branched, or cross-linked structure, conformation, isomerization, or chemical modification, that are capable of binding a virus vector in a physiologically reversible manner. Exemplary chemical modifications include methylation (Bello et al., 1985, J. Biomol. Struct. Dyn. 2:899-913) and glycosylation (Martinez-Fong et al., 1994, Hepatology 20:1602-1608). Such modifications can be made before or after synthesis of the polylysine.

In alternative embodiments of the compositions and methods of the invention, the virus-binding agent is a polycationic polypeptide other than a polylysine. Several amino acids are known to be positively charged at human physiological blood pH. Among the naturally occurring amino acids, lysine, arginine, and histidine are positively charged in this pH range. Other, naturally occurring and synthetic amino acids are positively charged in this pH range. Any of these amino acids can be polymerized into linear, branched, or cross-linked chains to generate polycationic polypeptides which are useful as virus-binding agents in the compositions and methods described herein. Synthetic polypeptides can be produced by either chemical synthetic

methods or recombinant methods. These polycationic polypeptides can be homopolymers, such as polylysine, polyarginine, polyornithine, or polyhistidine, or they can be heteropolymers, such as proteins such as myelin basic protein.

One particularly useful group of virus-binding agents are protein-ligand pairs. Examples of such pairs include antibodies (or antibody fragments such as Fab and Fab_2 fragments) which bind with discrete epitopes, cell surface proteins which bind with their corresponding viral envelope proteins (e.g. HIV gp120 and CD4), and proteins which bind with non-proteinaceous compounds (e.g. biotin and an avidin such as streptavidin). If either the protein or the ligand of a protein-ligand pair is present at the exterior surface of the matrix, then a virus vector which comprises the other member of the protein-ligand pair. Exemplary antibody-antigen pairs include antibodies which bind with surface epitopes of the virus vector. Numerous surface epitopes are known for most viruses, and antibodies thereto can be prepared routinely. Exemplary antibodies which bind with adenovirus vectors are those which bind with the hexon region of adenovirus coat protein and those which bind with the adenovirus knob protein (e.g. those described in Roivainen et al., 1998, Circulation 98:2534-2537 and Blackwell et al., 1999 Arch. Otolaryngol. Head Neck Surg. 125:856-863). The utility of the latter antibody (i.e. anti-knob) is counter-intuitive. The knob protein of adenovirus mediates binding of the virus to a host cell, entry into the cell, or both. The skilled worker would expect that antibodies which bind specifically with the knob protein would inactivate the virus (i.e. prevent an adenovirus vector from transfecting a target cell). Surprisingly, however, this antibody was discovered to bind adenovirus vector to the surface of a matrix in a physiologically reversible manner, such that the vector transfected cells to which the matrix was provided. These results demonstrate that the virus vector can be bound at the exterior surface of a matrix in a physiologically reversible manner using an antibody which binds specifically with substantially any surface epitope of the vector, including an epitope involved in cell binding or one involved in cell entry.

The virus-binding agents which are useful in the compositions and methods described herein are not limited to the compounds listed above, or any other particular set of molecules. Any molecule that has the property of binding the virus

vector to the exterior surface of the matrix is within the scope of the virus-binding agents described herein. Thus, for example, the virus-binding agent can be selected from the group consisting of a polycation, a polyanion, a cross-linking compound, a polypeptide which specifically binds with the virus vector, the protein of a specifically-binding protein-ligand pair, and the ligand of a specifically-binding protein-ligand pair. Exemplary polycations include polylysine, polyarginine, polyornithine, polyhistidine, myelin basic protein, a low molecular weight glycopeptide, a cationic amphiphilic alpha-helical oligopeptide having a repeating sequence, a histone, a galactosylated histone, polybrene, spermine, spermidine, prolamine, polyethylenimine, putrescine, cadaverine, and hexamine. An example of a polyanion is a nucleic acid. Exemplary cross-linking compounds include di-sulfhydryl compounds, N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), di-aldehyde compounds, and glutaraldehyde groups, and any other molecules which have the property of binding to both the matrix and the virus vector.

The virus-binding agent can optionally have another molecule bound to it, such as a polypeptide or a linker for the attachment of a polypeptide. In particular, this polypeptide can be an agent that imparts to the composition the ability to target and bind specific tissues or cells, allow them be retained at the administration site, etc., as described herein. The polypeptide can be attached, for example, by a disulfide linkage or by the known biotin-avidin system. If the virus-binding agent is also a polypeptide, the two molecules can also be part of the same peptide chain, bound by a standard peptide bond. For example, it can be desirable to create hybrid molecules combining a receptor-specific molecule and the virus-binding agent (e.g. Sosnowski et al., 1996, J. Biol. Chem. 271:33647-33653). The receptor-specific molecule can, for example, be an antibody, a fragment of an antibody of one of the types known in the art (e.g. an FAB fragment, as described in Janda et al., 1997, Science 275:945-948), a hormone, a growth factor, or any other molecule which imparts target specificity. Virus-binding agents, and other molecules bound thereto, should be non-toxic with respect to cells of the animal to which the composition is to be delivered, should be selected to exclude agents which reduce or alter the ability of the virus vector to enter cells, and should

furthermore be selected to permit release of the virus vector from the composition described herein *in vivo*.

Other virus-binding agents which are contemplated for use in the compositions and methods described herein include naturally-occurring proteins, such as those described herein, which have a net positive or negative charge at human physiologic blood pH and homopolymers and heteropolymers comprising a plurality of amino acid residues which are positively or negatively charged in this pH range. Exemplary amino acid residues which are negatively charged in this pH range include aspartate and glutamate, although the use of non-naturally-occurring and chemically modified amino acid residues is also contemplated. Polymers having a plurality of positively or negatively charged groups can also be effectively used as virus-binding agents.

A contemplated class of virus-binding agents includes proteins and corresponding ligands which form protein-ligand pairs. For example, a matrix can be made using a precursor comprising at least a portion of the FGF receptor binding site conjugated with a biopolymer such as purified type I collagen. This matrix could be bound with a recombinant virus vector which comprises at least a portion of FGF protein conjugated with a virus vector coat protein. Likewise, other such matrix-vector pairs which are useful to make the compositions described herein include antibody-antigen pairs and avidin-biotin pairs (e.g. streptavidin-biotin). When an antibody-antigen pair is used, the antigen is preferably an antigen which is present on the surface of the virus vector (i.e. expression of a recombinant fusion protein on the surface of the virus vector is not necessary), and the antibody linked with the matrix. For example, if the virus vector is an adenovirus vector, the antibody can be a previously described adenovirus-specific antibody (e.g. McHugh et al., 1988, J. Clin. Microbiol. 26:1957-1961; Cleveland et al., 1979, J. Immunol. Meth. 29:369-386).

The virus binding agent can also be a hybrid virus binding agent which comprises all of, or at least the virus-binding portions of, two or more virus binding agents. By way of example, hybrid virus binding agents include polycationic or polyanionic polymers having the protein or the ligand of a protein-ligand pair (e.g. an antibody-antigen pair or an avidin-biotin pair) linked thereto. The virus binding agents

(or virus-binding portions thereof) can be covalently or non-covalently linked, and are preferably covalently linked. The hybrid virus binding agent can be incorporated, as such, into the matrix of the composition. Alternatively, one or all virus binding agents can be incorporated into the matrix and the linkage between the two or more virus agents to yield the hybrid virus binding agent can be formed during or after formation of the matrix. As will be understood by the skilled artisan in light of the present disclosure, the hybrid virus binding agent can comprise all of or the virus-binding portions of two or more virus binding agents described herein or developed hereafter.

Implantable Devices

The compositions described herein can be used to make, to make a part of, to coat, or to coat at least a part of substantially any device which is to be applied to a surface of the body of an animal or which is to be inserted within the body of an animal. The implantable device can be one which is made and used for the sole purpose of delivering the composition to the animal, or the device can be one which is applied to the surface of or inserted within the body of the animal for a purpose other than merely delivering the composition to the animal. By way of example, the implantable device can be a plurality of microspheres which consist of the composition and which are implanted into the body animal for the sole purpose of delivering the virus vector to the animal. Further by way of example, the implantable device can be a cardiovascular stent coated with the composition; the stent is implanted within an artery of an animal both to maintain the patency of the artery and to deliver the composition to the intimal tissue of the artery or to other tissue in fluid communication with the lumen of the artery.

The compositions and methods described herein can be used to coat virtually any medical device. The coated devices provide a convenient means for local administration of the virus vector of the composition. For example, the compositions can be used to coat degradable and non-degradable sutures, orthopedic prostheses such as supporting rod implants, joint prostheses, pins for stabilizing fractures, bone cements and ceramics, tendon reconstruction implants, ligament reconstruction implants, cartilage substitutes, prosthetic implants, cardiovascular implants such as heart valve prostheses, pacemaker components, defibrillator components, angioplasty

devices, intravascular stents, acute and in-dwelling catheters, ductus arteriosus closure devices, implants deliverable by cardiac catheters such as atrial and ventricular septal defect closure devices, urologic implants such as urinary catheters and stents, neurosurgical implants such as neurosurgical shunts, ophthalmologic implants such as lens prosthesis, thin ophthalmic sutures, and corneal implants, dental prostheses, internal and external wound dressings such as bandages and hernia repair meshes, and other devices and implants, as will be apparent to the skilled artisan.

In one preferred embodiment, the device having a surface coated with the composition described herein is a suture or a wound dressing, such as a bandage, a film, a mesh, or a suspension of particles, microspheres, or nanospheres. Preferably, these devices are coated with a polymeric matrix having a virus vector bound to the surface thereof in a physiologically reversible manner, wherein the virus vector comprises a nucleic acid that stimulates wound healing *in vivo*. Sutures which can be coated in accordance with the methods and compositions described herein include any suture of natural or synthetic origin. Typical suture materials include, by way of example and not limitation, silk, cotton, linen, polyolefins such as polyethylene and polypropylene, polyesters such as polyethylene terephthalate, homopolymers and copolymers of hydroxycarboxylic acid esters, plain or chromicized collagen, plain or chromicized catgut, and suture substitutes such as cyanoacrylates. The sutures can take any convenient form such as braids or twists, and can have a wide range of sizes, such as are commonly employed in the art. Likewise, the bandages, films, and meshes can be substantially any of those presently employed as wound dressings, as well as any such dressings which are hereafter developed. The nucleic acid of the virus vector is preferably an expression construct encoding a wound healing therapeutic protein. The wound healing therapeutic protein can, for example, be selected from the group consisting of TGF-β, FGF, PDGF, PDGF-β, IGF, M-CGF, BMP, GH, and PTH.

In another preferred embodiment of the implantable device, the matrix of the composition is a stainless steel vascular stent and the virus vector comprises a nucleic acid selected from the group consisting of an expression construct encoding an anti-restenotic protein and an anti-restenotic antisense oligonucleotide. The expression construct can, for example, encode an anti-restenotic protein selected from the group

consisting of TPA, TGF-β, FGF, Rb, p21, and TK. The anti-restenotic antisense oligonucleotide can, for example, be selected from the group consisting of a c-*myb* antisense oligonucleotide, a c-*myc* antisense oligonucleotide, and a PCNA antisense oligonucleotide. The virus vector can be present on a surface of the stent together with the virus-binding agent described herein, or it can be present on a surface of the stent in the absence of a virus-binding agent other than the matrix (e.g. a negatively charged virus vector which binds to an inherently positively charged stainless steel stent in a physiologically reversible manner).

Implantable devices such as particles, patches, and bulk materials can be fashioned from the composition described herein either by making such an implantable device entirely from the composition (e.g. an implantable particle consisting of the composition), or by separately making the implantable device and coating all or a portion thereof with the composition (e.g. a stainless steel vascular stent having a polymeric coating comprising the composition). When the matrix of the composition comprises a polymer, the composition can be made by providing monomers and thereafter polymerizing the monomers (e.g. coating a device with matrix monomers, polymerizing the monomers, and thereafter binding the virus vector at the exterior surface of the matrix in a physiologically reversible manner). Alternatively, when the matrix of the composition described herein comprises a polymer, the composition can be made by providing the polymer dissolved or suspended in a solvent and thereafter removing the solvent (e.g. coating a device with a solvent in which a polymer is dissolved or suspended, removing the solvent, and thereafter binding the virus vector at the exterior surface of the matrix in a physiologically reversible manner). When implantable polymeric particles are made, the particles can either be synthesized as discrete particles, or the particles can be made by generating a bulk material and subsequently cutting, crushing, or grinding the bulk material to yield particles. Where bulk material comprising the composition described herein is made, it is contemplated that the bulk material can be cut, shaped, sliced, or otherwise fashioned to be adapted to a cavity or other bodily structure of an animal into which the composition is to be implanted. For example, implantation of slices of a bulk polymeric material comprising an anti-cancer agent near the site of a brain tumor in a human patient is

known (Fung et al., 1998, Cancer Res. 58:672-684; Brem et al., 1995, J. Neurooncol. 26:111-123). By way of example, the bulk polymeric material can comprise virus vector comprising either an expression construct encoding an anti-oncogenic protein or an anti-oncogenic antisense oligonucleotide.

Methods of Making the Compositions Described Herein

To make the compositions described herein, it is necessary to bind a virus vector to at least the exterior surface of a matrix in a physiologically reversible manner. The virus vector can be present only at the surface of the matrix or it can be present throughout the matrix. The virus vector can be bound at the exterior of the matrix directly, it can be bound to the exterior surface of the matrix by way of a virus-binding agent, or a portion of the virus vector can be bound directly at the exterior of the matrix and the remainder bound to the exterior surface of the matrix by way of a virus-binding agent.

In order to make a composition wherein the virus vector is bound directly to the exterior surface of the matrix, it is necessary to select a matrix to which the virus vector will bind in a physiologically reversible manner. By way of example, when the virus vector is positively or negatively charged at the pH at which the composition is to be made, the matrix can be a negatively or positively charged, respectively, material such as a polyanionic or polycationic polymeric matrix. When a negatively charged virus vector (e.g. an adenovirus vector) is contacted with a positively charged matrix (e.g. stainless steel in an aqueous liquid having a pH of 7.4), the virus vector will adhere to the exterior surface of the matrix. Further by way of example, the virus vector can be directly bound to the exterior surface of the matrix by reacting a reactive chemical group on the surface of the matrix with the same or a different reactive chemical group on the surface of the virus vector. For instance, if the matrix has, or is derivatized to have, sulfhydryl groups present at its surface and the virus vector comprises one or more proteins at its surface having a sulfhydryl group, the sulfhydryl groups of the vector and the matrix can be linked under oxidizing conditions, using known chemical reactions (e.g. using SPDP, as described herein). If necessary, one or more of the vector, the matrix, and the virus-binding agent can first be subjected to mild reducing conditions in order to reduce any disulfide bonds which

can exist on their surfaces, for example by incubating the vector, matrix, or agent in an oxygen-depleted solution comprising dithiothreitol or another sulfhydryl-reducing agent. The disulfide bonds formed between any two of the vector, the matrix, and the agent are cleaved under the reducing conditions present at many physiological locations, whereby the virus is released from the surface of the matrix. Of course, any bond-forming chemical reaction between a group on the surface of the matrix and a group on the surface of the virus vector (e.g. binding between biotin and an avidin such as streptavidin) can be used to bind the vector to the matrix, so long as the bond formed thereby can be cleaved or otherwise disrupted (e.g. by ionic displacement) under physiological conditions. Of course, if the matrix is biodegradable, it is not necessary that the bond between the matrix and the virus vector be capable of cleavage under physiological conditions, since degradation of the matrix will release the virus vector from the exterior surface of the matrix.

In order to make a composition wherein the virus vector is bound at the exterior surface of the matrix by means of a virus-binding agent, it is necessary to bind the virus vector to a virus-binding agent which is present on or at the exterior surface of the matrix or to bind the virus vector with a virus-binding agent which is subsequently bound at the exterior surface of the matrix. The virus-binding agent can, for example, be a polymer which coats, is suspended in, or is intermixed with the matrix, a chemical compound which is bound to a chemical group at the surface of the matrix, a chemical which is capable of binding to a chemical group at the surface of the matrix, a di-sulfhydryl compound such as SPDP, or a di-aldehyde compound such as glutaraldehyde. Of course, a plurality of compounds can be used to bind a virus vector to the exterior surface of the matrix. For example, a virus-binding agent can be bound with the virus vector, and the exterior surface of the matrix can be reacted with a second chemical, after which the second chemical and the virus-binding agent are bound to one another. The reaction sequence may also be reversed. For example, a collagen matrix can be derivatized using a sulfhydryl-containing reagent such as SPDP. An antibody which binds specifically with the desired virus vector can be bound with the sulfhydryl moieties of the derivatized collagen matrix under oxidizing conditions to bind the antibody with the matrix. Thereafter, the virus vector can be bound with the

antibody to bind it, in a physiologically reversible manner, at the exterior surface of the matrix. Unless matrix is biodegradable, however it is necessary that a covalent or non-covalent bond which links the virus vector to the exterior surface of the matrix be cleavable under the physiological conditions of the site to which the composition is to be administered.

In a preferred method of making the composition described herein, the matrix is a PLGA copolymer, the virus-binding agent is a polylysine polymer suspended in the PLGA copolymer, and the virus vector is an adenovirus vector. The PLGA copolymer is obtained from a commercial source or synthesized using standard methods, and is suspended in a solvent such as chloroform. The polylysine polymer is added to the suspension in order to intermix the PLGA and polylysine polymers. Optionally, the suspension is emulsified to form microspherical or nanospherical polylysine-containing PLGA particles upon removal of the solvent. The resulting matrix has polylysine present at the exterior surface thereof. The matrix is suspended in an aqueous solution such as pH 7.4 phosphate-buffered saline and then mixed with a suspension of the adenovirus vector. The negatively charged adenovirus vector binds with the positively charged polylysine present at the surface of the matrix in a physiologically reversible manner. When the virus-bound matrix is placed into a physiological setting, such as at a cardiovascular tissue of a human being, the adenovirus vector is released from the matrix in a sustained manner, whereby the adenovirus vector is capable of transfecting a cell with which the matrix is in fluid communication, such as an vascular intimal endothelial cell of the human located near the site at which the matrix was administered.

The composition can optionally be made to contain a plurality of layers of biodegradable matrix, each layer having a virus vector bound at the exterior surface thereof in a physiologically reversible manner. Each layer is made as described herein, another layer of matrix is deposited on the exterior surface of the previous layer of matrix and again made as described herein. This layering process can be repeated once, twice, five times, twenty times, fifty times, or any number of times to yield a multi-layer composition. Following release of the virus vector from the exterior surface of the outermost layer of matrix, the outermost layer of matrix biodegrades,

thereby exposing the exterior surface of the underlying layer, from which the virus vector can be released, after which the matrix can biodegrade, and so on.

In one embodiment, the matrix comprising the virus vector is provided in the form of microspheres or nanospheres. Methods for generating microspheres and nanospheres have been described (e.g. Song et al., 1997, J. Controlled Release 43:197-212; Gasper et al., 1998, J. Control. Rel. 52:53-62; Zambaux et al., 1998, J. Control. Rel. 50:31-40). However, the matrix can also be provided in other forms, including as a bulk matrix which can be sliced, cut, shaped, or otherwise manipulated prior to contacting the matrix with the virus vector as described herein.

The duration of the period during which the virus vector is contacted with the exterior surface of the matrix is not critical. The matrix can be contacted with the virus vector only momentarily, or the two can be contacted for a prolonged period of minutes, hours, days, or even weeks. The amount of virus vector which is bound at the exterior surface of the matrix can be changed, for example, by altering the chemical identity, the charge density, or the concentration of the virus-binding agent at the exterior surface of the matrix, by using a virus vector having a greater or lesser affinity for the virus-binding agent, by altering the conditions under which the matrix and the virus vector are contacted, or by some combination of these.

After contacting the matrix with the virus vector or with both the virus vector and the virus-binding agent, any excess virus vector can optionally be removed from the matrix, for example by rinsing the matrix with a solvent in which the virus vector can be suspended. Preferably, the solvent does not comprise a physiological level of ions, so that virus vector bound at the exterior surface of the matrix in a physiologically reversible manner is not caused to dissociate therefrom. Alternatively, any excess vector can be left in place on the exterior surface of the matrix to provide an initial bolus of vector upon administration of the composition.

Implantation of slices, particles, needles, strips, or other geometrical forms generated from a bulk material comprising the composition described herein is contemplated. The virus vector of the composition can be bonded to the exterior surface of the matrix either prior to reformation of the bulk material (e.g. by contacting the bulk material with the virus vector, or with both the virus vector and the virus-

binding agent, and thereafter reforming the bulk material, such as by slicing it) or after reformation of the bulk material (e.g. by making particles, slices, cubes, etc. from the bulk material, and thereafter contacting the particles, slices, or cubes with the virus vector, or with both the virus vector and the virus-binding agent).

Methods of Delivering a Virus Vector to an Animal Tissue

The compositions described herein are useful for delivering a virus vector to an animal tissue, particularly to a soft tissue of an animal. The virus vector is delivered to the tissue by placing a composition described herein which comprises the virus vector in fluid communication with the animal tissue. The composition can, for example, be in the form of implantable particles or an implantable device having a surface coated with the composition. Because the virus vector is bound to the matrix in a physiologically reversible manner, virus vector is released from the matrix upon administration of the composition in fluid communication with the tissue. Depending, for example, upon the affinity or chemical stability of the linkage between the virus-binding agent and the virus vector, the number and thickness of virus-bound matrix layers, and/or the rate of degradation of the matrix, the period of time over which the virus vector is released from the matrix can be controlled, thereby permitting either short-term (e.g. hours or days) or long-term (e.g. days, weeks, months, years, or even longer) sustained delivery of the virus vector to the tissue.

According to this method, the composition of the invention must be placed in fluid communication with the animal tissue to which the virus vector is to be delivered. The composition, by itself or attached to an implantable device, can be implanted into the animal at a location at which a liquid (e.g. a body fluid such as blood, lymph, cerebrospinal fluid, a mucosal secretion, stomach or intestinal contents, or amniotic fluid) contacts both the implanted composition and the tissue to which the virus vector is to be delivered. Preferably, the composition is implanted at a body location which is geometrically close to the tissue to which delivery is desired, preferably within a few centimeters of, within a few millimeters of, or even in contact with the desired tissue.

The method of placing the composition, or a particle or device comprising the composition, in fluid communication with the tissue to which the virus

vector is to be delivered is not critical. Virtually any method can be used which will result in placement of the composition in fluid communication with the tissue. By way of example, depending on the location of the tissue, the composition can be administered orally, injected, implanted, placed into an incision made in the animal body, embedded in an animal tissue (e.g. a vascular tissue undergoing balloon angioplasty) by stretching the tissue and pressing particles of the composition against the tissue, infusing the composition, sealing an incision made in the animal body using a suture, staple, or other device comprising or coated with the composition, or by applying the composition topically to an animal tissue.

The identity of the animal tissue to which the virus vector is delivered is not critical, except insofar as the identity of the tissue corresponds to the condition to be treated in the animal and the transfection capacity of the virus vector. In an important embodiment, the tissue is a soft tissue. By way of example, prevention or amelioration of restenosis in an animal generally requires delivery of the virus vector to a portion of a blood vessel of the animal, and promotion of wound healing in an animal generally requires delivery of the virus vector to the wounded tissue. Of course, a virus vector capable of transfecting cells of the desired tissue should be used in the composition described herein. An animal tissue can be treated either *in situ* (i.e. while the tissue remains a part of the animal body), or the tissue can be treated *in vitro* (i.e. after removing the tissue from the animal body, optionally before returning the tissue to the animal body or treating a tissue which has been cultured since removing the tissue from the animal body). Delivery of the virus vector using the compositions and methods described herein results in enhanced site-specific transfection efficiency, compared with existing virus vector delivery methods, which generally involve injection of virus vector suspended in a buffer.

It is known that diabetic patients experience impair wound healing ability, relative to non-diabetic patients. In one contemplated virus vector delivery method, a composition described herein is used to deliver a virus vector which comprises an expression construct encoding a wound healing therapeutic protein to a wounded tissue of an animal such as a diabetic human patient. For example, the wound healing therapeutic protein can be PDGF-β. The composition can, for example,

be administered to the patient in the form of polymeric particles, bulk material, or film having an adenovirus vector comprising the expression vector bound at the exterior surface thereof. The composition is administered directly to the site of the wound. If the wound is at the surface of the patient's body, the composition can, for example, be administered topically in the form of a wound dressing coated with the composition or in the form of a suspension of microspherical or nanospherical particles consisting of the composition. If the wound is beneath the patient's skin, the composition can, for example, be administered by injection of a suspension of particles of the composition or of a bulk material into the site of the wound, or by making an incision in the patient's skin to expose the wound site and then topically administering the composition to the wound site. Advantageously, an animal model exists which can be used to optimize the composition prior to administering the composition to a human patient. Db/db (diabetic) mice exhibit wound healing impairment which is characteristic of the wound healing impairment exhibited by human diabetic patients (Igel et al., 1996, Diabetologia 39:758-765). Methods of making db/db mice have been described (Prochazka et al., 1986, Diabetes 35:725-728). Use of this animal model to optimize the composition is described herein in Example 5.

Delivery of therapeutic compounds to ischemic tissues can be hindered by the limited blood supply which, by definition, is provided to ischemic tissues. In another contemplated virus vector delivery method, a composition described herein is used to deliver a virus vector to cells of an ischemic tissue in an animal such as a human patient. A composition described herein is administered directly to an ischemic tissue site in the animal in order to deliver a virus vector which comprises or contains a nucleic acid encoding, for example, at least one of PDGF, VEGF, and FGF. Advantageously, an animal model exists which can be used to optimize the composition prior to administering the composition to a human patient. A rabbit ear model of ischemic has been described, wherein the ischemic tissue in the rabbit ear model exhibits the characteristics of human ischemic tissue (Zhao et al., 1994, Arch. Surg. 129:1043-1049; Pierce et al., 1991, Am. J. Pathol. 138:629-646). Use of this animal model to optimize the composition is described herein in Example 6.

It is known that wounds in gastrointestinal tissues are slow to heal and that complications related to such wounds frequently develop. Reconstructive surgery involving gastrointestinal tissues is frequently necessary in patients afflicted with intestinal or colon cancers, colitis, or congenital malformations. In yet another contemplated virus vector delivery method, a composition described herein is administered intraluminally, periadventitially, or both, to an injured gastrointestinal tissue in an animal such as a human patient. Preferably, the virus vector of the composition comprises or contains a nucleic acid encoding, for example, at least one of PDGF, TGF-β, and FGF. Advantageously, an animal model exists which can be used to optimize the composition prior to administering the composition to a human patient. A rat ischemic intestinal anastomosis model of wounded gastrointestinal tissue has been described, wherein the intentionally wounded gastrointestinal tissue in the rat model exhibits the characteristics of wounded human gastrointestinal tissue (Hogstrom et al., 1986, Surgery 99:716-720). Use of this animal model to optimize the composition is described herein in Example 7.

The desirability of effective *in utero* gene therapy methods cannot be underestimated. Effective *in utero* gene therapy can be used to cure or alleviate genetically-based disease prior to the birth of the patient afflicted with the disease, thereby precluding significant discomfort or suffering. In still other contemplated virus vector delivery methods described herein, a composition described herein is administered *in utero* to an embryo or fetus of an animal such as a human. Such methods can have two goals: to induce immune tolerization of the animal toward the virus vector and/or to deliver a therapeutic composition to the animal *in utero*. Induction of immune tolerance toward the virus vector *in utero* permits the virus vector to be used for gene therapy of the animal without inducing an immune response toward the vector. The gene therapy can be performed while the animal is *in utero* or later in the animal's life, such as during the animal's adult life. *In utero* gene therapy methods have been described (e.g. Flake et al., 1996, N. Engl. J. Med. 335:1806-1810). In one embodiment of an *in utero* gene therapy method, a virus vector comprising a normal copy of the CFTR gene is delivered to airway cells of a fetus or embryo diagnosed as having one or more abnormal copies of this gene. Other genes which can be

advantageous employed in such an *in utero* gene therapy method include the genes encoding hemoglobin beta chain (for therapy of hemoglobinopathies) or cyclo-oxygenase (to enhance responsiveness for therapy). Likewise, substantially any localized congenital malformation can be treated *in utero* using a site specific virus vector-mediated gene delivery method such as those described herein. Advantageously, an animal model exists which can be used to optimize the composition prior to administering the composition to a human patient. Administration of a composition described herein to a sheep *in utero* in order to effect immune tolerization of the sheep to the virus vector can be used as a model of immune tolerization of human embryos and fetuses *in utero*. Use of this animal model to optimize the composition is described herein in Example 8.

The invention encompasses the preparation and use of pharmaceutical compositions comprising the a matrix having a virus vector bound at the exterior surface thereof in a physiologically reversible manner. Such a pharmaceutical composition can consist of the matrix alone, in a form suitable for administration to a subject, or the pharmaceutical composition can comprise the matrix or a particle or device coated with the matrix described herein combined with one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. Administration of one of these pharmaceutical compositions to a subject is useful for delivering the virus vector to the subject, as described elsewhere in the present disclosure.

As used herein, the term "pharmaceutically acceptable carrier" means a chemical composition with which the matrix described herein can be combined and which, following the combination, can be used to administer the virus vector to a subject.

The formulations of the pharmaceutical compositions described herein can be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the composition described herein into association with a carrier (e.g. water or phosphate-buffered saline) or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include humans and other primates, mammals including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs, birds including commercially relevant birds such as chickens, ducks, geese, and turkeys, fish including farm-raised fish and aquarium fish, and crustaceans such as farm-raised shellfish.

Pharmaceutical compositions that are useful in the methods described herein can be prepared, packaged, or sold in formulations suitable for oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, ophthalmic, or another route of administration. Other contemplated formulations include microspheres, nanospheres, projected nanoparticles, liposomal preparations, resealed erythrocytes comprising the composition, and immunologically-based formulations.

A pharmaceutical composition can be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the composition described herein. The amount of the composition is generally equal to an amount which contains a desirable dosage or amount of the virus vector for delivery to the subject or a convenient fraction of such a dosage such as one-half or one-third of such a dosage.

The relative amounts of the composition, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of

example, the composition described herein can comprise between 0.1% and 100% (w/w) of the pharmaceutical composition.

In addition to the composition described herein, a pharmaceutical composition can further comprise one or more additional pharmaceutically active agents.

A formulation of a pharmaceutical composition suitable for oral administration can be prepared, packaged, or sold in the form of a discrete solid dose unit including a tablet, a hard or soft capsule, a cachet, a troche, or a lozenge, each containing a predetermined amount of the composition described herein or a predetermined amount of the virus vector described herein. Other formulations suitable for oral administration include a powdered or granular formulation, an aqueous or oily suspension, or an emulsion.

As used herein, an "oily" liquid is one which comprises a carbon-containing liquid molecule and which exhibits a less polar character than water.

A tablet comprising the composition described herein can, for example, be made by compressing or molding the composition, optionally with one or more additional ingredients. Compressed tablets can be prepared by compressing, in a suitable device, the composition in a free-flowing form such as a microspherical or nanospherical powder or granular preparation, optionally mixed with one or more of a binder, a lubricant, an excipient, a surface active agent, and a dispersing agent. Molded tablets can be made by molding, in a suitable device, a mixture of the composition, a pharmaceutically acceptable carrier, and at least sufficient liquid to moisten the mixture. Pharmaceutically acceptable excipients used in the manufacture of tablets include inert diluents, granulating and disintegrating agents, binding agents, and lubricating agents. Known dispersing agents include potato starch and sodium starch glycolate. Known surface active agents include sodium lauryl sulfate. Known diluents include calcium carbonate, sodium carbonate, lactose, microcrystalline cellulose, calcium phosphate, calcium hydrogen phosphate, and sodium phosphate. Known granulating and disintegrating agents include corn starch and alginic acid. Known binding agents include gelatin, acacia, pre-gelatinized maize starch,

polyvinylpyrrolidone, and hydroxypropyl methylcellulose. Known lubricating agents include magnesium stearate, stearic acid, silica, and talc.

Tablets can be non-coated or they can be coated using known methods to achieve delayed disintegration in the gastrointestinal tract of a subject, thereby providing sustained release and absorption of the composition described herein. By way of example, a material such as glyceryl monostearate or glyceryl distearate can be used to coat tablets. Further by way of example, tablets can be coated using methods described in U.S. Patents numbers 4,256,108; 4,160,452; and 4,265,874 to form osmotically-controlled release tablets. Tablets can further comprise a sweetening agent, a flavoring agent, a coloring agent, a preservative, or some combination of these in order to provide pharmaceutically elegant and palatable preparation.

Hard capsules comprising the composition described herein can be made using a physiologically degradable composition, such as gelatin. Such hard capsules comprise the composition, and can further comprise additional ingredients including, for example, an inert solid diluent such as calcium carbonate, calcium phosphate, or kaolin.

Soft gelatin capsules comprising the composition described herein can be made using a physiologically degradable composition, such as gelatin. Such hard capsules comprise the composition, which can be mixed with water or an oil medium such as peanut oil, liquid paraffin, or olive oil.

Liquid suspensions of a pharmaceutical composition described herein which are suitable for oral administration can be prepared, packaged, and sold either in liquid form or in the form of a dry product intended for reconstitution with water or another suitable vehicle prior to use.

Liquid suspensions can be prepared using conventional methods to achieve suspension of the composition in an aqueous or oily vehicle. Aqueous vehicles include, for example, water and isotonic saline. Oily vehicles include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin. Liquid suspensions can further comprise one or more additional ingredients including suspending agents, dispersing or wetting agents, emulsifying agents, demulcents,

preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions can further comprise a thickening agent. Known suspending agents include sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, and cellulose derivatives such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose. Known dispersing or wetting agents include naturally-occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (e.g. polyoxyethylene stearate, heptadecaethyleneoxycetanol, polyoxyethylene sorbitol monooleate, and polyoxyethylene sorbitan monooleate, respectively). Known emulsifying agents include lecithin and acacia. Known preservatives include methyl, ethyl, or n-propyl-para- hydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and cetyl alcohol.

Powdered and granular formulations of a pharmaceutical preparation described herein can be prepared using known methods. Such formulations can be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations can further comprise one or more of dispersing or wetting agent, a suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, can also be included in these formulations.

A pharmaceutical composition described herein can be prepared, packaged, or sold in a formulation suitable for rectal administration. Such a composition can be in the form of, for example, a suppository, a retention enema preparation, and a suspension for rectal or colonic irrigation.

Suppository formulations can be made by combining the composition described herein with a non-irritating pharmaceutically acceptable excipient which is solid at ordinary room temperature (i.e. about 20°C) and which is liquid at the rectal

temperature of the subject (i.e. about 37°C in a healthy human). Suitable pharmaceutically acceptable excipients include cocoa butter, polyethylene glycols, and various glycerides. Suppository formulations can further comprise various additional ingredients including antioxidants and preservatives.

Retention enema preparations or suspensions for rectal or colonic irrigation can be made by combining the composition described herein with a pharmaceutically acceptable liquid carrier. As is known in the art, enema preparations can be administered using, and can be packaged within, a delivery device adapted to the rectal anatomy of the subject. Enema preparations can further comprise various additional ingredients including antioxidants and preservatives.

A pharmaceutical composition described herein can be prepared, packaged, or sold in a formulation suitable for vaginal administration. Such a composition can be in the form of, for example, a suppository, an impregnated or coated vaginally-insertable material such as a tampon, a douche suspension, or a suspension for vaginal irrigation.

Methods for impregnating or coating a material with a chemical composition are known in the art, and include methods of depositing or binding a chemical composition onto a surface, methods of incorporating a chemical composition into the structure of a material during the synthesis of the material (i.e. such as with a physiologically degradable material), and methods of absorbing an aqueous or oily solution or suspension into an absorbent material, with or without subsequent drying.

Douche preparations or suspensions for vaginal irrigation can be made by combining the composition described herein with a pharmaceutically acceptable liquid carrier. As is known in the art, douche preparations can be administered using, and can be packaged within, a delivery device adapted to the vaginal anatomy of the subject. Douche preparations can further comprise various additional ingredients including antioxidants, antibiotics, antifungal agents, and preservatives.

Vaginal preparations of the composition described herein can also be used for administration *in utero* of the virus vector described herein to an ovum, embryo, fetus, or to a neonate during birth. Such preparations are preferably placed in the uterus of the woman bearing the ovum, embryo, fetus, or neonate, although such

preparations can also be placed cervically or vaginally or can be physically contacted with the embryo or fetus or on or within the chorionic or amniotic membranes.

As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, by application using a device (e.g. a balloon angiocatheter) inserted at one site in a blood vessel of an animal and physically urged along the vessel to a second site in the blood vessel of the animal, by administration of the composition using a wound dressing (e.g. a bandage, a suture, or a hernia repair mesh) comprising the pharmaceutical composition, and the like. In particular, parenteral administration is contemplated to include subcutaneous, intraperitoneal, intravenous, intraarterial, intramuscular, or intrasternal injection and intravenous, intraarterial, or kidney dialytic infusion techniques.

Formulations of a pharmaceutical composition suitable for parenteral administration comprise the composition described herein combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations can be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations can be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative. Formulations for parenteral administration include suspensions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations can further comprise one or more additional ingredients including suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e. microspherical or nanospherical powder or granular) form for reconstitution with a suitable vehicle (e.g. sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition. Use of a double-balloon or 'sweating' balloon type of angiocatheter to deliver a pharmaceutical

composition described herein to the intimal surface of a blood vessel of an animal is contemplated. Also contemplated is delivery of the virus vector described herein using a pharmaceutical composition comprising a wound dressing which comprises the composition described herein.

The pharmaceutical compositions can be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension. This suspension can be formulated according to the known art, and can comprise, in addition to the composition described herein, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations can be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the composition described herein in bulk form, in particulate form, in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Compositions for sustained release or implantation can comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

Formulations suitable for topical administration include liquid or semi-liquid preparations such as liniments, lotions, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes, and solutions or suspensions. Topically-administrable formulations can, for example, comprise from about 1% to about 10% (w/w) of the composition described herein, although the concentration of the composition in the solvent of the formulation can be higher. Formulations for topical administration can further comprise one or more of the additional ingredients described herein. Preferably, the composition is in a microspherical or nanospherical form when it is used to generate a pharmaceutical composition for topical administration, as these forms can be more efficiently taken up by animal tissues.

A pharmaceutical composition described herein can be prepared, packaged, or sold in a formulation suitable for pulmonary administration via the buccal

cavity. Such a formulation can comprise dry particles which comprise, which consist of, or which are coated with the composition described herein and which have a diameter in the range from about 0.5 to about 7 nanometers, and preferably from about 1 to about 6 nanometers. Such compositions are conveniently in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant can be directed to disperse the powder or using a self-propelling solvent/powder-dispensing container such as a device comprising the composition described herein dissolved or suspended in a biocompatible, low-boiling propellant in a sealed container. Preferably, such powders comprise particles wherein at least 98% of the particles by weight have a diameter greater than 0.5 nanometers and at least 95% of the particles by number have a diameter less than 7 nanometers. More preferably, at least 95% of the particles by weight have a diameter greater than 1 nanometer and at least 90% of the particles by number have a diameter less than 6 nanometers. Dry powder compositions preferably include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

Low boiling propellants generally include liquid propellants having a boiling point of below 65°F at atmospheric pressure. Generally the propellant can constitute 50 to 99.9% (w/w) of the pharmaceutical composition, and the composition can constitute 0.1 to 20% (w/w) of the pharmaceutical composition. The propellant can further comprise additional ingredients such as a liquid non-ionic or solid anionic surfactant or a solid diluent (preferably having a particle size of the same order as particles comprising the active ingredient).

Pharmaceutical compositions formulated for pulmonary delivery can also provide the composition described herein in the form of droplets of a suspension of the composition. Such formulations can be prepared, packaged, or sold as aqueous or dilute alcoholic suspensions, optionally sterile, comprising the active ingredient, and can conveniently be administered using any nebulization or atomization device. Such formulations can further comprise one or more additional ingredients including a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, or a preservative such as methylhydroxybenzoate. The droplets provided

by this route of administration preferably have an average diameter in the range from about 0.1 to about 200 nanometers.

The formulations described herein as being useful for pulmonary delivery are also useful for intranasal delivery of a pharmaceutical composition described herein.

Another formulation suitable for intranasal administration is a coarse powder comprising the composition described herein and having an average particle from about 0.2 to 500 micrometers. Such a formulation is administered in the manner in which snuff is taken i.e. by rapid inhalation through the nasal passage from a container of the powder held close to the nares.

Formulations suitable for nasal administration can, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of the composition described herein, and can further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition can be prepared, packaged, or sold in a formulation suitable for buccal administration. Such formulations can, for example, be in the form of tablets or lozenges made using conventional methods, and can, for example, 0.1 to 20% (w/w) of the composition described herein, the balance comprising an orally dissolvable or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternatively, formulations suitable for buccal administration can comprise a powder or an aerosolized or atomized suspension comprising the composition. Such powdered, aerosolized, or aerosolized formulations, when dispersed, preferably have an average particle or droplet size in the range from about 0.1 to about 200 nanometers, and can further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition can be prepared, packaged, or sold in a formulation suitable for ophthalmic administration. Such formulations can, for example, be in the form of eye drops including, for example, a 0.1-1.0% (w/w) suspension of the composition described herein in an aqueous or oily liquid carrier. Such drops can further comprise buffering agents, salts, or one or more other of the additional ingredients described herein. Other ophthalmalmically-administrable

formulations which are useful include those which comprise the active ingredient in microcrystalline form or in a liposomal preparation.

As used herein, "additional ingredients" include one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other "additional ingredients" which can be included in the pharmaceutical compositions described herein are known in the art and described, for example in Genaro, ed., 1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, which is incorporated herein by reference.

It is understood that the ordinarily skilled physician or veterinarian will determine and prescribe an effective amount of the composition described herein for delivery to the subject. In so proceeding, the physician or veterinarian can, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. It is further understood, however, that the specific dose level for any particular subject will depend upon a variety of factors including the identity of the virus vector described herein, the identity and properties of the nucleic acid carried by the virus vector, the estimated efficiency of uptake of the virus vector by the subject, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the severity of the condition being treated.

The Kits of the Invention

The invention includes a variety of kits which are useful for making and using the compositions described herein.

In one embodiment of a kit of the invention, the kit comprises the matrix of the composition described herein and an instructional material which describes binding a virus vector at the exterior surface of the matrix in a

physiologically reversible manner. The matrix can, for example, be supplied together with a virus-binding agent, either as a separate component of the kit or as a matrix comprising the virus-binding agent at at least the exterior surface thereof. The matrix can, for example, be provided in the form of a non-polymer matrix which can be mixed or coated with the virus vector, monomers which can be combined with the virus vector, with the virus-binding agent, or with both, and thereafter polymerized. monomers which can be polymerized and thereafter combined with the virus vector, with the virus-binding agent, or with both, and the like. The instructional material can, for example, describe how to use the components of the kit, optionally with commercially available reagents or with a virus vector of the user's design, to generate a composition described herein. Alternatively, or in addition, the instructional material can describe how to use the composition described herein to deliver a virus vector to an animal tissue. The kit can optionally comprise one or more of the virus vector described herein, a virus vector precursor, the virus-binding agent described herein, and a solvent in which the matrix described herein can be dissolved or suspended. In an alternative embodiment, the kit comprises the composition described herein and one or more of an instructional material for administering the composition to an animal tissue, a device for administering the composition to an animal tissue, and pharmaceutical carrier for combination with the composition prior to administering the composition to an animal tissue.

Definitions

As used herein, each of the following terms has the meaning associated with it in this section.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

As used herein, a "virus vector" is a nucleic acid-containing composition which comprises a protein which naturally occurs in a virus, wherein the composition is capable of transferring its nucleic acid into the interior of at least one type of cell when the virus vector is contacted with the cell.

A "virus vector precursor" is a virus vector into which a nucleic acid can be introduced using a known method. For example, a virus vector precursor comprising a nucleic acid having a multiple restriction site enables a user to create a virus vector of interest by inserting a nucleic acid of interest at the multiple restriction site.

A "transfection indicator" is a component of a virus vector which, when transferred to a cell by the virus vector, confers an observable phenotype to the cell. A non-limiting example of a transfection indicator is a β-galactosidase gene operably linked to a promoter.

By "nucleic acid" is meant any homopolymer or heteropolymer of deoxyribonucleosides, ribonucleosides, or nucleoside analogs. The nucleotide analogs can be any compound known in the art to be or subsequently discovered to be useful as a structural analog of a ribonucleoside or a deoxyribonucleoside. Nucleotide analogs include nucleotides comprising bases other than the five biologically occurring bases (adenine, guanine, thymine, cytosine and uracil). The monomers of the nucleic acid can be connected by phosphodiester linkages or modified linkages such as phosphotriester, phosphoramidate, siloxane, carbonate, carboxymethyl ester, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, bridged phosphoramidate, bridged phosphoramidate, bridged methylene phosphonate, phosphorothioate, methylphosphonate, phosphorodithioate, bridged phosphorothioate or sulfone linkages, and combinations of such linkages.

A nucleic acid "expression construct" is a nucleic acid which encodes an RNA or protein product which is formed upon transcription or upon transcription and translation, respectively, of the nucleic acid. RNA expression constructs which can be directly translated to generate a protein product, or which can be reverse transcribed and either transcribed or transcribed and translated to generate an RNA or protein product, respectively, are also included within this definition.

A "transfection construct" is a nucleic acid-containing vector which comprises at least one component of a virus, whereby, by the action of the virus component, the vector is enabled to enter a cell, to integrate its nucleic acid into the

genome of the cell, or to transcribe, translate, or reverse-translate its nucleic acid in the cell.

A "transcribable construct" is a DNA molecule having a transcriptional start site and any promoter/regulatory sequence which is necessary to enable an RNA molecule to be generated by transcription thereof or an RNA molecule having any promoter/regulatory sequence which is necessary to enable generation of a DNA molecule by reverse transcription thereof.

By describing two polynucleotides as "operably linked" is meant that a single-stranded or double-stranded nucleic acid moiety comprises the two polynucleotides arranged within the nucleic acid moiety in such a manner that at least one of the two polynucleotides is able to exert a physiological effect by which it is characterized upon the other. By way of example, a promoter operably linked to the coding region of a gene is able to promote transcription of the coding region.

As used herein, the term "promoter/regulatory sequence" means a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulator sequence. In some instances, this sequence can be the core promoter sequence and in other instances, this sequence can also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence can, for example, be one which expresses the gene product in a tissue specific manner.

A "ribozyme" is an RNA molecule, or a molecule comprising an RNA molecule and a polypeptide molecule, which is capable of specifically catalyzing a chemical reaction, in a manner analogous to enzymatic catalysis.

An "antisense oligonucleotide" is a nucleic acid molecule (e.g. DNA, RNA, or a polymer comprising one or more nucleotide analogs), at least a portion of which is complementary to a nucleic acid which is present in a cell. The antisense oligonucleotides preferably comprise between about twelve and about fifty nucleotides. More preferably, the antisense oligonucleotides comprise between about fourteen and about thirty nucleotides. Most preferably, the antisense oligonucleotides comprise between about sixteen and about twenty-one nucleotides. The antisense oligonucleotides include phosphorothioate oligonucleotides and other modifications of

oligonucleotides, as described herein. Methods for synthesizing oligonucleotides, phosphorothioate oligonucleotides, and otherwise modified oligonucleotides are known in the art (U.S. Patent No: 5,034,506; Nielsen et al., 1991, Science 254: 1497), and each of these types of modified oligonucleotides in included within the scope of the invention.

A "therapeutic protein" is a protein which, when provided to or expressed in a diseased or wounded tissue, alleviates, prevents, or inhibits the disease, promotes healing of the wound, or prevents worsening of the wound.

A "viral genome fragment" means at least a portion of a nucleic acid which is a component of a naturally-occurring virus.

A "gene fragment" means at least a portion of a nucleic acid which, alone or in conjunction with other operably linked nucleic acids, constitutes a gene.

A "matrix" is any material at the exterior surface of which a virus vector can be bound in a physiologically reversible manner, either directly or by means of a virus-binding agent.

Virus vectors are bound at a surface "in a physiologically reversible manner" if they are covalently or non-covalently bonded to the surface, either directly or by means of a virus-binding agent, and if, when the surface is subjected to an environment in which animal cells are capable of surviving (e.g. a cell culture medium or the surface or interior of an animal body), at least a fraction of the virus vectors dissociate from the surface over time. By way of example, the rate of dissociation can be between 0.1% and 100% of bound vectors per day in pH 7.4 phosphate-buffered saline. The rate of dissociation should be controlled such that the body region in fluid communication with the surface is not flooded with vector immediately. Release of the vector from the surface preferably continues at least as long as cells in the region need to be modified. For example, it is advantageous that less than 5%, and not more than 30% of the virus bound to the surface is released therefrom during the first 24 hours following delivery of the surface in fluid communication with the body region. It is also advantageous that substantially complete release of the virus vector from the surface occurs not later than from about 7 (e.g. for treatment of inflammation) to 180 days (e.g. for treatment of tumors) or to 9 months (e.g. for *in utero* applications).

The "exterior surface" of a matrix, device, particle, or surface is the surface or portion of a surface which contacts a solvent in which the matrix, device, particle, or surface is immersed.

A compound is present "at" the exterior surface of a matrix if the compound is present on the exterior surface of the matrix, of if the compound is present within the matrix at a position which is located geometrically near enough to the exterior surface that it can be contacted (e.g. by convection or by diffusion) by a solvent in which the matrix is suspended.

A compound is present "substantially only at the exterior surface" of a matrix if at least most of the compound is present at the exterior surface of the matrix and no more than less than most of the compound is present in an interior portion of the matrix. Preferably, at least 75%, preferably at least 90%, and more preferably at least 99% of the compound is present at the exterior surface of the matrix, and less than 25%, preferably less than 10%, and more preferably less than 1% is present at an interior portion of the matrix.

The "interior portion" of a matrix is a portion of the matrix which does not contact a solvent in which the matrix is suspended or in which a device or particle coated with the matrix is suspended or immersed, at least until the matrix has at least partially biodegraded. It is understood that, in instances in which multiple layers of matrix are present, the "interior portion(s)" of the matrix can refer only to the innermost portion of the innermost layer of the matrix (i.e. the first-deposited layer) or to the inner portion of each layer of the matrix. The interior portion of the matrix does not include the exterior surface of the matrix, but can include any and all parts of the matrix that are not exposed on the exterior surface.

A material is "biocompatible" with respect to an animal if the presence of the material in the animal is not injurious to the animal. By way of example, a biocompatible material does not induce an immune response to the material when the material is implanted in the body of an animal.

A material is "biodegradable" if the material undergoes decomposition when contacted with a biological system such upon implantation into an animal. The decomposition can be evidenced, for example, by dissolution, depolymerization,

disintegration, or by another chemical or physical change whereby the bulk of the material in the biological system is reduced over time. Such decomposition can be, but is not necessarily, catalyzed by a component of the biological system (e.g. an enzyme).

A material is "in fluid communication" with a tissue if the material is in contact with a fluid which normally contacts the tissue, either *in vitro* or *in vivo*. Examples of materials in fluid communication with a tissue include a material deposited, suspended, or dissolved in a tissue culture medium in which the tissue is maintained, a material deposited, suspended, or dissolved in a body fluid which normally contacts the tissue in an animal, and a material which physically contacts the tissue. For the purposes of the present disclosure, embryonic and fetal tissues are considered to be "in fluid communication" with materials which physically contact the embryo or fetus, with materials which are deposited, suspended, or dissolved in amniotic fluid which surrounds the embryo or fetus, and with materials which are deposited, suspended, or dissolved in uteral, cervical, or vaginal fluids of an animal which bears the embryo or fetus.

A "soft tissue" means a tissue which does not primarily consist of one or more precipitated inorganic minerals. By way of example, mammalian bones and teeth primarily consist of a variety of precipitated inorganic minerals, and are not soft tissues.

A device, particle, or surface is "coated" with a material if at least a part of a surface of the device or particle or at least a part of the surface has the material present at the exterior surface thereof.

As used herein, the term "virus-binding agent" and grammatical forms thereof generally refers to molecules which are capable of covalently or non-covalently binding with a virus vector.

A "cross-linking" compound is a chemical compound which is capable of binding to both a matrix of the composition described herein and a virus vector.

A "di-sulfhydryl" compound is a chemical compound having at least two sulfhydryl substituents. Dithiothreitol is an example of a di-sulfhydryl compound.

A "di-aldehyde" compound is a chemical compound having at least two aldehyde substituents. Glutaraldehyde is an example of a di-aldehyde compound.

A "protein-ligand pair" refers to a protein and another molecule, wherein the protein specifically binds with the other molecule. Examples of protein-ligand pairs include an antibody and its corresponding epitope and an avidin protein, such as streptavidin, and biotin.

A "particle" or "particulate formulation" of a matrix means a matrix having geometric dimensions compatible with injection, cellular ingestion, or mucous membrane penetration. Thus, such a matrix typically comprises, or preferably consists essentially of, spherical or ellipsoid particles having a maximal geometric dimension of about 50 microns, preferably less than about one micron, and more preferably, from about 100 nanometers to 500 nanometers.

A "bulk material" or "bulk formulation" of a matrix means a monolithic matrix, having geometric dimensions in excess of those compatible with injection, cellular ingestion, or mucous membrane penetration. Such bulk formulations typically have one or more geometric dimensions in excess of 50 microns in diameter. Bulk materials can, for example, be provided in the form of spheres, irregular shapes, sheets, needles, bars, and the like.

The "hydrodynamic diameter" of an object such as a molecule or a particle refers to the diameter of an imaginary sphere which is traced by rotating the object in all directions around its center of mass. The hydrodynamic diameter can be thought of roughly as the 'effective size' of an object rotating rapidly in space or in solution. By way of example, the hydrodynamic diameter of a sphere is the actual diameter of the sphere, and the hydrodynamic diameter of a rigid rod-shaped object is the length of the object along its longest axis (i.e. the length of the rod).

An "implantable device" means a particle or other object which can be entirely or partially inserted into the body of an animal. Implantable devices thus include particles which, when applied topically to a surface of the animal body, are capable of being taken up by a tissue or cell of the animal. The means by which the particle or other object is inserted into the animal body is not critical, and includes, for example, swallowing, inhalation, injection, topical application, physical penetration, insertion into an incision made in the animal body, and the like.

A "wound healing therapeutic protein" is a protein which, when provided to a wounded tissue in an animal, promotes healing of the wounded tissue.

An "anti-restenotic protein" is a protein which, when provided to the site of an intimal vascular injury (e.g. following performance of a balloon angioplasty procedure at the site), prevents, inhibits, or alleviates restenotic injury at the site. The anti-restenotic protein can, for example, prevent migration of smooth muscle or other cells to the site, prevent proliferation of smooth muscle or other cells at the site, or cause smooth muscle or other cells to dissociate from the site.

An "anti-restenotic antisense oligonucleotide" is an antisense oligonucleotide which, when provided to the site of an intimal vascular injury, prevents, inhibits, or alleviates restenotic injury at the site.

An "oncogene" as used herein, includes both genes which are identified in the art as oncogenes and those which are identified as tumor suppressor genes. A distinguishing characteristic of both oncogenes and tumor suppressor genes is their association with control the processes of oncogenesis, metastasis, or apoptosis.

An "anti-oncogenic protein" is a protein, such as a protein encoded by an oncogene, which, when provided to the site of a cancerous or pre-cancerous lesion in an animal, prevents, inhibits, or reverses abnormal cellular growth at the site or induces apoptosis of cells of the lesion.

An "anti-oncogenic antisense oligonucleotide" is an antisense oligonucleotide which, when provided to the site of cancerous or pre-cancerous lesion in an animal, prevents, inhibits, or reverses abnormal cellular growth at the site or induces apoptosis of cells of the lesion.

As used herein, an "instructional material" includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the composition described herein for delivering a virus vector according to the methods described herein or which can be used to communicate a method of making or using a composition as described herein. The instructional material can, for example, be affixed to a container which contains the composition or be shipped together with a container which contains the composition. Alternatively, the instructional material can be shipped separately from the container with the

intention that the instructional material and the composition be used cooperatively by the recipient.

The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only and the invention should in no way be construed as being limited to these Examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Example 1

In this Example, fluorescently labeled, negatively charged polystyrene nanoparticles were used as a detectable model of adenovirus particles. The nanoparticles which were used were Fluoresbrite™ Carboxylate Microsphere YG particles (fluorescein isothiocyanate- {FITC-} labeled microspheres, Polysciences, Inc., Warrington, PA). The nanoparticles had a diameter of 0.50 micrometers and a ζ-potential of -33.65 ± 1.77 millivolts. An aqueous suspension of 10 milligrams per milliliter poly-L-lysine (PLL) having a molecular weight of 3970 was dispersed into a suspension of 25 milligrams per milliliter polylactate-polyglycolate copolymer (PLGA) in chloroform. The ratio of the volume of the PLL suspension to the volume of the PLGA suspension was 1:6. Dispersion of the PLL suspension into the PLGA suspension formed an emulsion. The emulsion was used to coat an arterial stent (Crown stent, Cordis Inc., Miami, FL). The PLL/PLGA coated stent was air dried at room temperature (i.e. about 20°C) for twenty-four hours.

The dried, coated stent was incubated in a suspension of Fluoresbrite™ Carboxylate Microsphere YG particles for about fifteen minutes, and then rinsed extensively with water. The microsphere-coated stent was examined under a fluorescent microscope fitted with an FITC filter. High affinity binding of fluorescently labeled microspheres was observed on the PLL/PLGA coated stent, relative to a stent subjected to the same treatment but coated only with PLGA.

The PLL/PLGA coated stent having fluorescently labeled microspheres bound thereto was incubated in pH 7.4 phosphate buffered saline for 13 hours, and then in a 1 molar sodium chloride solution for 13 hours. Following these incubations, the stent was again examined by fluorescence microscopy, and little or no loss of the

fluorescently labeled microspheres was observed, relative to the number of fluorescently labeled microspheres present on the stent prior to these incubations. This stent was then incubated in pH 7.4 Tris/EDTA buffer containing 0.1% (w/v) sodium dodecyl sulfate (SDS). After seven days of incubation in this buffer, only slight reduction in the number of fluorescently labeled microspheres bound to the stent was observed and the coating on the stent remained significantly fluorescent.

The experiments described in this Example demonstrate that negatively charged particles which are comparable to adenovirus can be bound to the surface of an object coated with a matrix comprising a polycationic virus-binding agent. These experiments furthermore demonstrate that such particles are released from the surface over a prolonged period of time.

Example 2

The experiments described in this Example demonstrated that a virus vector comprising an expression construct could be bound to the surface of an object coated with a matrix having a virus-binding agent present at the exterior surface thereof, and that the vector transfected cells which were in fluid communication with the matrix. Both the cells and the matrix were geometrically fixed in these experiments; thus, these experiments demonstrated that the virus vector was released from the surface. In these experiments, the virus vector which was used was a replication-defective recombinant type 5 human adenovirus comprising an expression construct which comprised a nuclear-targeted nucleic acid encoding β-galactosidase operably linked to a CMV promoter.

A glass cover slip was coated with 100 microliters of a (3-50% (w/v)) solution comprising PLGA having a molecular weight range of 5000 to 120,000, 1-20% (w/v) PLL, and chloroform. The cover slip was lyophilized overnight and then incubated with a suspension of approximately 10^{10} to 10^{12} particles of the virus vector in phosphate buffered saline, or with PBS alone as a negative control. Non-bound virus vector was removed by repeatedly rinsing the cover slip with PBS at room temperature. The material on the cover slip was fixed in methanol, dried at room temperature, and then re-hydrated in PBS. The cover slips were incubated with a suspension of a mouse monoclonal IgG which binds specifically to the virus vector,

and then incubated with a suspension of a goat anti-mouse FITC-conjugated IgG diluted in PBS. Irrelevant mouse IgG was applied to parallel samples to serve as an additional negative control. Immunofluorescence microscopy revealed significant and specific fluorescence associated with cover slips which were coated with the matrix and contacted with the virus vector, as indicated in Figure 1A, but no significant or specific fluorescence associated with control cover slips, as indicated in Figure 1B.

Next, to determine the ability of the virus vector to transfect cells after having been bound to the matrix, A10 vascular smooth muscle cells were cultured in Medium 199 (a commonly known and commercially available medium) containing 10% fetal bovine serum on cover slips on which PLGA/PLL polymer-adenoviral complexes had been formed. Cells were fixed either in 10% (v/v) neutral buffered formalin for X-gal staining or in methanol for detection of the virus vector. Twenty-four hours following plating, high levels of β-galactosidase activity was detected in the nuclei of A10 cells located near the matrix, as indicated in Figure 1C. The number of cells which were stained with X-gal increased with increasing culture time, as indicated by comparing Figures 1C and 1D.

The experiments described in this Example demonstrate that the virus vector could be bound to the surface of an object coated with the matrix, and that the virus vector bound to the surface could be used to transfect cells in fluid communication with that surface.

Example 3

In the experiments described in this Example, a method of making a composition for controlled delivery of a virus vector according to the methods of the present invention is described. A method of characterizing the composition is also described.

A composition comprising a biodegradable polymeric matrix having a virus binding agent at the exterior surface thereof can be made as described herein in Example 1. The PLGA polymer used in this composition has an average molecular weight of about 120,000. Polylysine having an average molecular weight of about 4000 is used as the virus binding agent, and is incorporated into the PLGA polymer as described in Example 1. The emulsion formed by dispersing the polylysine suspension

in the PLGA suspension is deposited onto a surface such as the surface of a glass cover slip, to yield a film having a thickness of about 100 micrometers. The final proportion of the polylysine/PLGA film that is polylysine is, on a dry weight basis, from about 2% (w/w) to about 20% (w/w).

The capacity of the polylysine/PLGA film for binding virus vectors having a negative charge can be assessed, for example, using fluorescently labeled, negatively charged nanoparticles, as described in Example 1, using a fluorescently labeled antibody or another protein which specifically binds to a virus vector that is contacted with the film (e.g. an adenovirus vector such as that described in Example 2), or using a visualization method which detects delivery to cells of an expression construct carried by the virus vector (e.g. a recombinant adenovirus vector which comprises an expression construct encoding β-galactosidase, as in Example 2). The capacity of the polylysine/PLGA film to deliver a virus vector which comprises an expression construct encoding a therapeutic gene product such as platelet-derived growth factor-B (PDGF-β) can be assessed by binding such a virus vector to the surface of a polylysine/PLGA film and thereafter incubating the film in fluid communication with cells capable of being transfected by the virus vector. The ability of the film to confer the benefit of the therapeutic gene product on the cells is an indication that the film is useful for delivering the virus vector to the cells.

The compositions can be characterized under simulated physiologic conditions, i.e. in phosphate buffered saline at pH 7.4 and 37°C. The buffer can further comprise 0.1% (w/v) SDS in order to dissociate complexes formed by association of DNA with polylysine. The structural integrity (e.g. phase transition temperature) of the matrix can be assessed by observing any of several biophysical parameters of the matrix. These parameters include those which can be measured by known Fourier transform infrared spectroscopy and differential scanning calorimetry techniques. The results obtained using the experimental procedures described in the Example can be used to optimize the characteristics of the composition described herein in light of the virus vector to be used and the cells to which the virus vector is to be delivered.

Example 4

The experimental procedures described in this Example can be used to assess the efficiency with which a composition described herein delivers a virus vector to target cells. These methods can also be used to assess the duration of the period during which the composition will deliver the virus vector to the cells.

A composition described herein comprising a virus vector such as an adenovirus vector is incubated in fluid communication with cultured cells. In one embodiment of these experiments, the composition is placed in physical contact with a monolayer of cells (e.g. primary human dermal fibroblasts cells) growing on the surface of a culture plate. Infiltration of the cells into the matrix can be assessed by microscopic or other examination of the matrix. The kinetics of release of the virus vector and transfection of the cells can be assessed by examining the rate at which the cells are transfected and the number of cells which are transfected. The virus vector can be a virus which is itself cytotoxic or which comprises an expression construct encoding a protein which induces cell death, in which case transfection can be assessed, if the virus vector is replication competent, by counting plaques in the cell monolayer which result from death of cells. The virus vector can instead comprise a transfection indicator, such as an expression construct encoding β-galactosidase.

In a separate experiment, the composition can be suspended above the cell monolayer in order to differentiate transfection attributable to physical contact of the composition with the monolayer from transfection attributable to release of the virus vector from the matrix. Cell culture studies are preferably performed in triplicate.

When transfection of cells by a virus vector comprising an expression construct (e.g. one encoding PDGF-β) needs to be assessed, this assessment can be made using an ELISA assay using an antibody which specifically binds with the product encoded by the expression construct. Such transfection can also be assessed using Western blot methods using a similar antibody. If desired target cells obtained from an animal or cells of the same type as desired target cells in an animal are used in the experimental procedures described in this Example, then the results obtained using these procedures will help the skilled worker to predict an appropriate dosages of the

composition to administer to the animal in order to deliver the virus vector to desired target cells in the animal.

Example 5

The methods described in this Example relate to an experimental procedure for assessing the usefulness of a composition described herein for delivering a virus vector to wounded tissue of an animal for the purpose of promoting wound healing.

In these methods, diabetic mice are used as a model for virus vector delivery to excision-wounded tissue for the purpose of promoting healing of the tissue. The diabetic mice which are used are db/db (diabetic) mice, such as those described in Prochazka et al. (1986, Diabetes 35:725-728). Excision wounding was performed by excising a piece of skin from each mouse. Such mice exhibit impaired excisional wound healing ability, relative to normal mice. Excisional wounds are made upon individual db/db mice. Mesh (e.g. polyester mesh) coated with a matrix and having a virus-binding agent at the surface thereof and having a virus vector comprising an expression construct which encodes a wound healing protein (e.g. a recombinant, replication-defective adenovirus comprising an expression construct encoding PDGF-β) is used to dress the wounds of randomly selected wounded mice. Mesh coated with the same polymer and virus-binding agent, but not comprising the virus vector, is used to dress the wounds of the remaining (i.e. control) mice. Preferably, groups of 10 mice are compared. Excision wounded db/db mice can also be injected with the virus vector in order to compare the efficiency of virus vector delivery by direct injection with the efficiency of such delivery using the composition described herein.

At selected times after wound dressing, wound healing morphology is examined, including, for example, immunohistochemical demonstration of wound healing protein expression and *in situ* hybridization experiments to demonstrate persistence of the expression construct in the cells of the excision wounded tissue.

The experimental procedures described in this Example can be used to demonstrate delivery of a virus vector to wounded tissue in an animal.

Example 6

The usefulness of a composition described herein for delivering a virus vector to wounded tissue of an animal for the purpose of promoting wound healing can also be assessed using a rabbit ischemic injury model, as described (Zhao et al., 1994, Arch. Surgery 129:1043-1049; Pierce et al., 1991, Am. J. Pathol. 138:629-646). Compositions and virus vector formulations analogous to those described herein in Example 5 are used. Wound healing morphology, as described in Example 5, and the extent of re-vascularization of the wound site are assessed to determine the usefulness of the composition for delivering a virus vector to wounded tissue.

Example 7

The usefulness of a composition described herein for delivering a virus vector to wounded tissue of an animal for the purpose of promoting wound healing can also be assessed using a rat intestinal anastomosis model as follows. Two-month-old rats are subjected to laparotomy, and the colon of each is incised and anastomosed. A wedge of mesenteric vessels is ligated to make the area of the anastomosis ischemic. Under these condition, a near uniform anastomotic breakdown and leak of the enteric contents occurs in non-treated animals.

The laporotomized rats are divided into three groups: one group is treated with a composition comprising a virus vector which comprises an expression construct encoding a wound healing protein such as PDGF-β; a second group is treated with the same composition which does not comprise the virus vector; a third group is not treated. In treated rats, the composition is applied in the form of a film wrapped around the outside of the anastomosis. After two weeks the animals in each of the three groups are sacrificed. For each group of rats, survival, anastomotic healing, presence or absence of peritonitis, intestinal lumen patency, tensiometry, and evidence of wound healing protein expression are assessed to determine the efficacy of the composition described herein for delivering the virus vector to the wounded tissue.

Example 8

The usefulness of a composition described herein for delivering a virus vector to an animal *in utero* for the purpose of inducing immune tolerance of the virus

vector in the animal or for delivering the virus vector to cells or a tissue of the animal can be assessed using the experimental procedures described in this Example.

The composition comprises a virus vector comprising a transfection indicator. The composition is delivered to a fetal sheep at 65 days gestation by injection into the fetal peritoneum using a 22 gauge needle. Placement of the needle into the peritoneum is guided using an ultrasound or similar non-invasive imaging device. For comparison, an amount of the same virus vector approximately equivalent to the amount of vector in the composition is injected into a separate fetal sheep in the form of a suspension of the vector in a physiologically acceptable carrier.

The sheep are carried to term, and a total body survey for the transfection indicator is performed. The total body survey can be performed by performing PCR or reverse transcriptase- PCR using appropriate primers to detect a nucleic acid transfection indicator, or the survey can be performed by performing Western blotting or activity assays (e.g. a β-galactosidase activity assay) to detect a protein transfection indicator. Identification of the presence of the transfection indicator at one or more body locations in the full-term sheep is an indication that *in utero* administration of the virus vector effected transfection by the vector of cells at those body locations or their precursors.

The full-term sheep are also assayed for development of neutralizing antibodies to the virus vector or to a protein transfection indicator. These studies can be performed by administering either the virus vector or the protein transfection indicator to the sheep *post-partum*, and assessing whether an immune response is induced. The absence of induction of an immune response to *post-partum* administration of the virus vector or the protein transfection indicator is an indication that *in utero* administration of the virus vector induced immune tolerization of the virus vector or the protein transfection indicator.

Example 9

In this Example, a hybrid virus binding agent is used to bind a virus vector (e.g. an adenovirus gene vector) at the exterior portion of a matrix. A hybrid virus binding agent is one which is capable of binding the virus vector by at least two mechanisms. In this Example, the mechanisms by which the hybrid virus binding

agent binds the virus vector include ionic attraction and protein-ligand pair interaction. Even though the virus vector is bound at the exterior surface of the matrix using two (or more) mechanisms, the virus vector is nonetheless bound in a biologically reversible manner. The virus vector can be similarly bound within the bulk of the matrix, but is preferably bound only at the exterior surface thereof.

The matrix in this Example comprises a PLGA polymer as described elsewhere in this disclosure and a hybrid virus binding agent. The hybrid virus binding agent comprises a polycation having the protein half of a protein-ligand pair linked thereto. In this example, the hybrid virus binding agent comprises polylysine having an antibody linked thereto, wherein the antibody binds specifically with the virus vector (an adenovirus gene vector in this Example). The antibody is bound to polylysine using a disulfide cross-linking agent (e.g. dithiothreitol) to link a sulfhydryl residue on the antibody, by way of the disulfide cross-linking agent, to a sulfhydryl residue on the polylysine. A sulfhydryl residue can be incorporated into the polylysine, for example, by including one or more cysteine residues in the polylysine peptide chain or by reacting the polylysine with a carbodiimide in the presence of cysteine. Once the antibody has been linked with the polylysine, the polylysine-antibody hybrid is capable of binding a negatively-charged virus vector (e.g. an adenovirus gene vector) both by way of interactions between the positively-charged lysine residue side chains with the negatively charged virus vector and by way of specific binding interactions between the antibody and its corresponding epitopes on the virus vector surface. By contacting the matrix comprising PLGA and the polylysine-antibody hybrid with a suspension of an adenovirus gene vector, the vector binds to at least the exterior surface of the matrix in a biologically reversible manner. Furthermore, because the virus vector is bound to the matrix in two or more different ways, bi- or multi-phasic release of the vector can be effected, due to differences in the rates of dissociation of the differently-bound forms of the virus from the matrix in a biological system.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention can

be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations

What is claimed is:

1. A composition for delivery of a virus vector to an animal cell, the composition comprising the virus vector and a matrix having an exterior surface, wherein the virus vector is bound at the exterior surface in a physiologically reversible manner.

2. The composition of claim 1, wherein the matrix is biodegradable and has an internal portion comprising the virus vector bound to the matrix in a physiologically reversible manner.

3. The composition of claim 1, wherein the virus vector is present substantially only at the exterior surface.

4. The composition of claim 1, wherein the matrix is not biodegradable and wherein the virus vector is bound to the matrix in a physiologically reversible manner.

5. The composition of claim 1, further comprising a virus-binding agent at the exterior surface, wherein the virus vector is bound to the virus-binding agent.

6. The composition of claim 5, wherein the matrix is biodegradable and has an internal portion comprising the virus vector bound to the virus-binding agent in a physiologically reversible manner.

7. The composition of claim 5, wherein the matrix is biodegradable.

8. The composition of claim 5, wherein the virus vector is bound to the virus-binding agent in a physiologically reversible manner.

9. The composition of claim 8, wherein the matrix is not biodegradable.

10. The composition of claim 8, wherein the virus vector has a net charge, and wherein the virus-binding agent has a second net charge opposite the net charge of the virus vector.

11. The composition of claim 9, wherein the virus-binding agent is selected from the group consisting of a polycation, a polyanion, a cross-linking compound, a polypeptide which specifically binds with the virus vector, the protein of a specifically-binding protein-ligand pair, and the ligand of a specifically-binding protein-ligand pair.

12. The composition of claim 11, wherein the polycation is selected from the group consisting of polylysine, polyarginine, polyornithine, polyhistidine, myelin basic protein, a low molecular weight glycopeptide, a cationic amphiphilic alpha-helical oligopeptide having a repeating sequence, a histone, a galactosylated histone, polybrene, spermine, spermidine, prolamine, polyethylenimine, putrescine, cadaverine, and hexamine.

13. The composition of claim 12, wherein the polycation is poly-L-lysine.

14. The composition of claim 11, wherein the polyanion is a nucleic acid.

15. The composition of claim 11, wherein the cross-linking compound is selected from the group consisting of a di-sulfhydryl compound, SPDP, a di-aldehyde compound, and glutaraldehyde.

16. The composition of claim 11, wherein the polypeptide which specifically binds with the virus vector is selected from the group consisting of an antibody which specifically binds with the virus vector, a fragment of an antibody which specifically binds with the virus vector, and a virus receptor protein.

17. The composition of claim 11, wherein the specifically-binding protein-ligand pair is selected from the group consisting of biotin and an avidin, an antibody and an epitope to which the antibody specifically binds, and a viral coat protein and a cell-surface molecule with which the viral coat protein specifically binds.

18. The composition of claim 1, wherein the matrix is selected from the group consisting of a charged biocompatible material, a biocompatible polymer, a biodegradable polymer, a biocompatible biodegradable polymer, polylactic acid, polyglycolic acid, polycaprolactone, a copolymer of polylactic acid and polyglycolic acid, a copolymer of polylactic acid and polycaprolactone, a copolymer of polyglycolic acid and polycaprolactone, a polygylcolide, a polyanhydride, a polyacrylate, a polyalkyl cyanoacrylate, n-butyl cyanoacrylate, isopropyl cyanoacrylate, a polyacrylamide, a polyorthoester, a polyphosphazene, a polypeptide, a polyurethane, a polystyrene, a polystyrene sulfonic acid, a polystyrene carboxylic acid, a polyalkylene oxide, a polyethylene, a polyvinyl chloride, a polyamide, a nylon, a polyester, a rayon, a polypropylene, a polyacrylonitrile, an acrylic, a polyisoprene, a polybutadiene, a polybutadiene-polyisoprene copolymer, a neoprene, a nitrile rubber, a polyisobutylene, an olefinic rubber, an ethylene-propylene rubber, an ethylene-propylene-diene monomer rubber, a polyurethane elastomer, a silicone rubber, a fluoroelastomer, a fluorosilicone rubber, a vinyl acetate homopolymer, a vinyl acetate copolymer, an ethylene vinyl acetate copolymer, an acrylates homopolymer, an acrylates copolymer, polymethylmethacrylate, polyethylmethacrylate, polymethacrylate, ethylene glycol dimethacrylate, ethylene dimethacrylate, hydroxymethyl methacrylate, a polyvinylpyrrolidone, a polyacrylonitrile butadiene, a polycarbonate, a polyamide, a fluoropolymer, polytetrafluoroethylene, polyvinyl fluoride, a polystyrene, a styrene acrylonitrile homopolymers, a styrene acrylonitrile copolymer, a cellulose acetate, an acrylonitrile butadiene styrene homopolymer, a acrylonitrile butadiene styrene copolymer, a polymethylpentene, a polysulfone, a polyester, a polyimide, a polyisobutylene, a polymethylstyrene, an alginate, an agarose, a dextrin, a dextran, a

multi-block polymer, a biocompatible metal alloy, titanium, platinum, stainless steel, hydroxyapatite, tricalcium phosphate, cocoa butter, a wax, and a ceramic material.

19. The composition of claim 18, wherein the matrix comprises a biodegradable polylactate/polyglycolate copolymer.

20. The composition of claim 1, wherein the matrix is not electrically conductive.

21. The composition of claim 1, wherein the matrix is in a form selected from the group consisting of a bulk material, a particle, a microsphere, a nanosphere, a device, a coating on a surface of a bulk material, a coating on a surface of a particle, a coating on a surface of a microsphere, a coating on a surface of a nanosphere, and a coating on a surface of a device.

22. The composition of claim 1, wherein the virus vector comprises a transfection indicator.

23. The composition of claim 22, wherein the transfection indicator is selected from the group consisting of a nucleic acid, a nucleic acid analog, a transcription construct, an antisense oligonucleotide, a ribozyme, and an expression construct.

24. The composition of claim 23, wherein the transfection indicator is selected from the group consisting of an expression construct encoding a wound healing therapeutic protein, an expression construct encoding an anti-restenotic protein, an expression construct encoding an anti-oncogenic protein, an anti-restenotic antisense oligonucleotide, and an anti-oncogenic antisense oligonucleotide.

25. The composition of claim 24, wherein the wound healing therapeutic protein is selected from the group consisting of TGF-β, FGF, PDGF, PDGF-β, IGF, M-CGF, BMP, GH, and PTH.

26. The composition of claim 24, wherein the anti-restenotic protein is selected from the group consisting of TPA, TGF-β, FGF, Rb, p21, and TK.

27. The composition of claim 24, wherein the anti-oncogenic protein is encoded by a gene selected from the group consisting of *abl*, *akt2*, *apc*, *bcl2α*, *bcl2β*, *bcl3*, *bcr*, *brca1*, *brca2*, *cbl*, *ccnd1*, *cdk4*, *crk-II*, *csf1r/fms*, *dbl*, *dcc*, *dpc4/smad4*, *e-cad*, *e2f1/rbap*, *egfr/erbb-1*, *elk1*, *elk3*, *eph*, *erg*, *ets1*, *ets2*, *fer*, *fgr/src2*, *fli1/ergb2*, *fos*, *fps/fes*, *fra1*, *fra2*, *fyn*, *hck*, *hek*, *her2/erbb-2/neu*, *her3/erbb-3*, *her4/erbb-4*, *hras1*, *hst2*, *hstf1*, *ink4a*, *ink4b*, *int2/fgf3*, *jun*, *junb*, *jund*, *kip2*, *kit*, *kras2a*, *kras2b*, *lck*, *lyn*, *mas*, *max*, *mcc*, *met*, *mlh1*, *mos*, *msh2*, *msh3*, *msh6*, *myb*, *myba*, *mybb*, *myc*, *mycl1*, *mycn*, *nf1*, *nf2*, *nras*, *p53*, *pdgfb*, *pim1*, *pms1*, *pms2*, *ptc*, *pten*, *raf1*, *rb1*, *rel*, *ret*, *ros1*, *ski*, *src1*, *tal1*, *tgfbr2*, *thra1*, *thrb*, *tiam1*, *trk*, *vav*, *vhl*, *waf1*, *wnt1*, *wnt2*, *wt1*, and *yes1*.

28. The composition of claim 24, wherein the anti-restenotic antisense oligonucleotide is selected from the group consisting of a c-*myb* antisense oligonucleotide, a c-*myc* antisense oligonucleotide, and a PCNA antisense oligonucleotide.

29. The composition of claim 24, wherein the anti-oncogenic antisense oligonucleotide is selected from the group consisting of an *abl* antisense oligonucleotide, an *akt2* antisense oligonucleotide, an *apc* antisense oligonucleotide, a *bcl2α* antisense oligonucleotide, a *bcl2β* antisense oligonucleotide, a *bcl3* antisense oligonucleotide, a *bcr* antisense oligonucleotide, a *brca1* antisense oligonucleotide, a *brca2* antisense oligonucleotide, a *cbl* antisense oligonucleotide, a *ccnd1* antisense oligonucleotide, a *cdk4* antisense oligonucleotide, a *crk-II* antisense oligonucleotide, a *csf1r/fms* antisense oligonucleotide, a *dbl* antisense oligonucleotide, a *dcc* antisense oligonucleotide, a *dpc4/smad4* antisense oligonucleotide, an *e-cad* antisense

oligonucleotide, an *e2f1/rbap* antisense oligonucleotide, an *egfr/erbb-1* antisense oligonucleotide, an *elk1* antisense oligonucleotide, an *elk3* antisense oligonucleotide, an *eph* antisense oligonucleotide, an *erg* antisense oligonucleotide, an *ets1* antisense oligonucleotide, an *ets2* antisense oligonucleotide, an *fer* antisense oligonucleotide, an *fgr/src2* antisense oligonucleotide, an *fli1/ergb2* antisense oligonucleotide, an *fos* antisense oligonucleotide, an *fps/fes* antisense oligonucleotide, an *fra1* antisense oligonucleotide, an *fra2* antisense oligonucleotide, an *fyn* antisense oligonucleotide, an *hck* antisense oligonucleotide, an *hek* antisense oligonucleotide, an *her2/erbb-2/neu* antisense oligonucleotide, an *her3/erbb-3* antisense oligonucleotide, an *her4/erbb-4* antisense oligonucleotide, an *hras1* antisense oligonucleotide, an *hst2* antisense oligonucleotide, an *hstf1* antisense oligonucleotide, an *ink4a* antisense oligonucleotide, an *ink4b* antisense oligonucleotide, an *int2/fgf3* antisense oligonucleotide, a *jun* antisense oligonucleotide, a *junb* antisense oligonucleotide, a *jund* antisense oligonucleotide, a *kip2* antisense oligonucleotide, a *kit* antisense oligonucleotide, a *kras2a* antisense oligonucleotide, a *kras2b* antisense oligonucleotide, an *lck* antisense oligonucleotide, an *lyn* antisense oligonucleotide, an *mas* antisense oligonucleotide, an *max* antisense oligonucleotide, an *mcc* antisense oligonucleotide, an *met* antisense oligonucleotide, an *mlh1* antisense oligonucleotide, an *mos* antisense oligonucleotide, an *msh2* antisense oligonucleotide, an *msh3* antisense oligonucleotide, an *msh6* antisense oligonucleotide, an *myb* antisense oligonucleotide, an *myba* antisense oligonucleotide, an *mybb* antisense oligonucleotide, an *myc* antisense oligonucleotide, an *mycl1* antisense oligonucleotide, an *mycn* antisense oligonucleotide, an *nf1* antisense oligonucleotide, an *nf2* antisense oligonucleotide, an *nras* antisense oligonucleotide, a *p53* antisense oligonucleotide, a *pdgfb* antisense oligonucleotide, a *pim1* antisense oligonucleotide, a *pms1* antisense oligonucleotide, a *pms2* antisense oligonucleotide, a *ptc* antisense oligonucleotide, a *pten* antisense oligonucleotide, an *raf1* antisense oligonucleotide, a *rb1* antisense oligonucleotide, an *rel* antisense oligonucleotide, an *ret* antisense oligonucleotide, an *ros1* antisense oligonucleotide, an *ski* antisense oligonucleotide, an *src1* antisense oligonucleotide, a *tal1* antisense oligonucleotide, a *tgfbr2* antisense oligonucleotide, a *thra1* antisense oligonucleotide, a *thrb* antisense oligonucleotide, a *tiam1* antisense oligonucleotide, a *trk* antisense oligonucleotide, a

vav antisense oligonucleotide, a *vhl* antisense oligonucleotide, a *waf1* antisense oligonucleotide, a *wnt1* antisense oligonucleotide, a *wnt2* antisense oligonucleotide, a *wt1* antisense oligonucleotide, and a *yes1* antisense oligonucleotide.

30. The composition of claim 1, wherein the virus vector is selected from the group consisting of an adenovirus vector, a retrovirus vector, an adeno-associated virus vector, and a herpes virus vector.

31. The composition of claim 30, wherein the virus vector is an adenovirus vector.

32. A surface coated with the composition of claim 1.

33. An implantable device having a surface coated with a composition comprising a virus vector and a matrix having an exterior surface, wherein the virus vector is bound at the exterior surface in a physiologically reversible manner.

34. The implantable device of claim 33, wherein the device is selected from the group consisting of a wound dressing, a suture, a particle, a vascular stent, and a bulk material.

35. The implantable device of claim 34, wherein the device is a vascular stent, wherein the matrix is a polylactate/polyglycolate copolymer comprising polylysine at the exterior surface, wherein the virus vector is bound with the polylysine, and wherein the virus vector comprises a transfection indicator selected from the group consisting of an expression construct encoding an anti-restenotic protein and an anti-restenotic antisense oligonucleotide.

36. The implantable device of claim 34, wherein the device is a suture, wherein the matrix is a polylactate/polyglycolate copolymer comprising polylysine at

the exterior surface, wherein the virus vector is bound with the polylysine, and wherein the virus vector comprises an expression construct encoding a wound healing protein.

37. The implantable device of claim 31, wherein the device is a particle, wherein the matrix is a polylactate/polyglycolate copolymer comprising polylysine at the exterior surface, wherein the virus vector is bound with the polylysine, and wherein the virus vector comprises a transfection indicator selected from the group consisting of an expression construct encoding a wound healing therapeutic protein, an expression construct encoding an anti-restenotic protein, an expression construct encoding an anti-oncogenic protein, an anti-restenotic antisense oligonucleotide, and an anti-oncogenic antisense oligonucleotide.

38. The implantable device of claim 37, wherein the particle has a diameter no greater than about 900 micrometers.

39. The method of claim 38, wherein the particle has a diameter no greater than about 1 micrometer.

40. The implantable device of claim 31, wherein the device is a bulk material, wherein the matrix is a polylactate/polyglycolate copolymer comprising polylysine at the exterior surface, wherein the virus vector is bound with the polylysine, and wherein the virus vector comprises a transfection indicator selected from the group consisting of an expression construct encoding a wound healing therapeutic protein, an expression construct encoding an anti-restenotic protein, an expression construct encoding an anti-oncogenic protein, an anti-restenotic antisense oligonucleotide, and an anti-oncogenic antisense oligonucleotide.

41. A surface coated with the composition of claim 5.

42. An implantable device having a surface coated with the composition of claim 5.

43. The implantable device of claim 42, wherein the device is selected from the group consisting of a wound dressing, a suture, a particle, a vascular stent, and a bulk material.

44. A method of making a composition for delivery of a virus vector to an animal, the method comprising contacting the virus vector with a matrix which has an exterior surface, whereby the virus vector binds at the exterior surface in a physiologically reversible manner.

45. The method of claim 44, the matrix comprises a virus-binding agent at the exterior surface.

46. The method of claim 44, wherein the matrix is biodegradable.

47. The method of claim 44, wherein the matrix is on a surface of an implantable device.

48. The method of claim 47, wherein a suspension comprising the matrix and a solvent is applied to the surface of the implantable device and the solvent is at least substantially removed from the surface prior to contacting the virus vector with the matrix.

49. The method of claim 48, wherein the suspension further comprises the virus-binding agent.

50. The method of claim 47, wherein a precursor composition comprising a plurality of monomers of the polymer is applied to the surface of the implantable device and the monomers are polymerized prior to contacting the virus vector with the matrix.

51. The method of claim 50, wherein the precursor composition further comprises the virus-binding agent.

52. A method of delivering a virus vector to an animal tissue, the method comprising placing in fluid communication with the animal tissue a composition comprising a matrix having an exterior surface, wherein the virus vector is bound at the exterior surface in a physiologically reversible manner.

53. The method of claim 52, wherein the composition further comprises a virus-binding agent at the exterior surface, and wherein the virus vector is bound to the virus-binding agent.

54. The method of claim 52, wherein the composition is placed in contact with the animal tissue.

55. The method of claim 52, wherein the animal tissue is outside of the body of the animal from which it was obtained.

56. The method of claim 52, wherein the animal tissue is in an animal.

57. The method of claim 52, wherein placing the composition in fluid communication with the tissue comprises placing in fluid communication with the tissue an implantable device having a surface coated with the composition.

58. The method of claim 57, wherein the device is selected from the group consisting of a wound dressing, a suture, a particle, a vascular stent, and a bulk material.

59. The method of claim 52, wherein the tissue is selected from the group consisting of a wounded tissue, an ischemic tissue, a gastrointestinal tissue, an embryonic tissue, and a fetal tissue.

60. The method of claim 52, wherein the animal is a human.

61. A kit comprising a matrix having an exterior surface, a virus-binding agent, and an instructional material which describes binding a virus vector at the exterior surface in a physiologically reversible manner.

62. The kit of claim 61, further comprising a virus-binding agent.

63. The kit of claim 62, wherein the virus-binding agent is present at the exterior surface.

64. The kit of claim 61, further comprising a virus vector precursor.

65. The kit of claim 64, wherein the virus vector precursor is an adenovirus vector comprising a nucleic acid having a multiple restriction site.

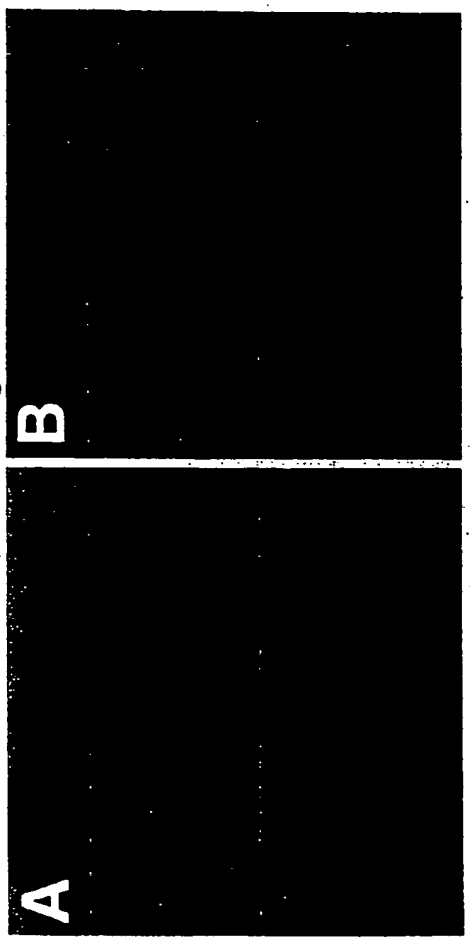

Fig. 1B

Fig. 1A

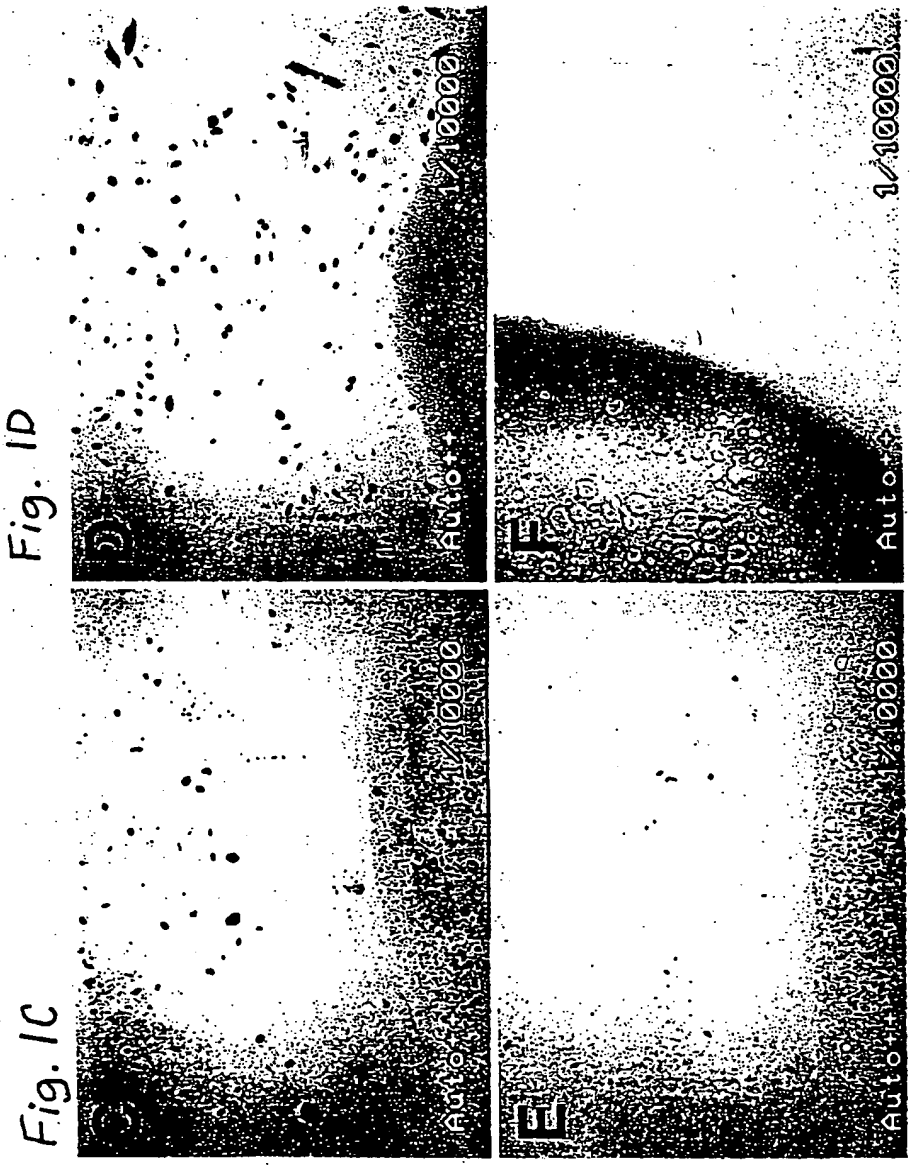

Fig. 1D

Fig. 1F

Fig. 1C

Fig. 1E

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/01193

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :A61K 48/00; C12N 15/63; A61B 19/00; C07H 21/04
US CL : 514/44; 435/320.1, 6; 128/849; 536/24.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/44; 435/320.1, 6; 128/849; 536/24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN, BIOSIS, MEDLINE, CAPLUS, LIFESCI, WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98/44143 A1 (GENZYME CORPORATION) 08 October 1998 (08.10.99) see entire document.	1-65
A,E	SEIMENS et al. Viral Vector Delivery in Solid-State Vehicles: Gene Expression in a Murine Cancer Model. Journal of the National Cancer Institute. 01 March 2000, Vol. 92, No. 5, pages 403-412, see entire document.	1-65
Y	YE et al. Cytokine Transgene Expression and Promoter Usage in Primary CD34+ Cells Using Particle-Mediated Gene Delivery. Human Gen Therapy. 10 October 1998, Vol. 9, pages 2197-2205, see entire document.	1-65
Y,P	WO 99/53903 A1 (THE REGENTS OF THE UNIVERSITY OF MICHIGAN) 28 October 1999 (28.10.99), see entire document.	1-65

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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16 MAY 2000

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/01193

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	WO 99/53943 A2 (ANGIOGENIX INCORPORATED) 28 October 1999 (28.10.99), see entire document.	1-65
Y	WO 97/47254 A1 (THE REGENTS OF THE UNIVERSITY OF MICHIGAN) 18 December 1997 (18.12.97), see entire document.	32-60
Y	LABHASETWAR et al. Gene based therapies for restenosis. Advanced Drug Delivery Reviews. 1997, Vol. 24, pages 109-120, see entire document.	24, 27-29,
Y	WO 96/20698 A2 (THE BOARD OF REGENTS: UNIVERSITY OF MICHIGAN) 11 JULY 1996 (11.07.96), see entire document.	1-65